FACTORS AFFECTING ESTUARINE POPULATIONS OF *NEREOCYSTIS LUETKEANA* IN KACHEMAK BAY, ALASKA

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A

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By

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ABSTRACT

Nereocystis luetkeana forms extensive kelp beds in Kachemak Bay, Alaska. Salinity and turbidity gradients apparently regulate kelp bed distribution throughout this estuary. The beds are large at the entrance of the bay, only solitary stands occur in the inner bay, and no kelp is found at the head of the bay. The role of salinity and turbidity on *Nereocystis* sporophyte growth was investigated by performing reciprocal transplants among three beds along the bay axis and regularly measuring stipe growth. The effects of salinity and light on spores were studied in the laboratory by recording sinking tendency, settlement success, germination success, and germ tube length under different salinity and light levels. Grazing effects of *Lacuna vincta* impacted the survival of *Nereocystis* transplants *in-situ* and on plants of different age classes in the laboratory. Overall, this study suggests a possible negative estuarine effect on sporophytes transplanted from the outer to the inner bay and on certain aspects of spore development. Herbivory pressure had significant localized effects on *Nereocystis* survival and was most pronounced on juvenile plants. The dynamics of *Nereocystis* kelp beds in Kachemak Bay results from large-scale environmental factors and local-scale biological processes.

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CHAPTER 1
GENERAL INTRODUCTION

1.1. - IMPORTANCE OF KELP FORESTS

Kelp forest communities are dominant near-shore, subtidal habitats that are important to the entire marine ecosystem because of their primary and secondary productivity (Duggins *et al.* 1989). Kelp forests are amongst the most productive ecosystems on earth; for example, primary productivity in *Macrocystis* kelp forests in California ranges from 350 to 1500 $gC/m^2/yr$ and *Laminaria* forests of the North Atlantic and South Africa can produce between 120 and 1900 $gC/m^2/yr$ (Mann 1982). In comparison, annual production rates of rain and temporal forests range from 400 to 1900 and 30 to 600 $gC/m^2/yr$, respectively (Valiela 1995). As primary producers, kelps fix carbon that is eventually passed to successive trophic levels. Kelps serve as a direct food source for grazers such as urchins, abalone, kelp crabs, and some herbivorous fish; but they also supply carbon to the detrital loop, as a substantial portion of the organic matter produced degrades into detritus and dissolved organic nutrients (Mann 1973; Duggins 1988; Duggins *et al.* 1989; Soares *et al.* 1997). As *Nereocystis luetkeana* blades elongate, the distal tip of the blade erodes and significantly contributes to the input of fixed carbon into kelp beds (Nicholson 1970; Clendenning 1971a). These forms of organic material can disperse extensively and supply the secondary production of temperate near-shore ecosystems several miles away from the source bed (Duggins *et al.* 1990). Some kelp fragments also drift away and provide food and shelter to organisms (such as amphipods) far from the original bed either in the open ocean or on a beach (North 1991; Kozloff 1996). The productivity and availability of phytoplankton also is thought to be enhanced within kelp beds and to become part of the kelp bed food web as those particles are consumed by filter feeders (North 1991).

Kelp forests are associated with a great diversity of organisms including seabirds, marine mammals, fish, and invertebrates (for review see, North 1971; Foster and Schiel 1985; North 1991). North (1971) compiled an extensive list of over 800 species of animals (ranging from protozoans to mammals) associated with the kelp beds of southern and Baja California. Kelp forests are prime habitats and feeding grounds for several seabirds such as Steller and common eiders (Bustnes and Loenne 1995; Bustnes and Systad 2001) and black and ruddy turnstones (Bradley and Bradley 1993). Sea otters also are predators of species that live in kelp forests (Estes and Palmisano 1974; Palmisano and Estes 1976; Duggins 1980; Ebeling and Laur 1988; DeWreede 1992; Estes *et al.* 1998; Konar 2000). Several studies suggest that macroalgae play a crucial role in the abundance, diversity, and distribution of fish (many of which are commercially and recreationally important) and their associated prey (Davies 1968; Miller and Geibel 1973; Russell 1977; Ebeling and Laur 1985; Bodkin 1986, 1988; Duggins 1988; DeMartini and Roberts

1990; Levin 1991; Shaffer and Parks 1994; Nybakken 1997). Macroalgal habitats are essential fish nursery grounds (Feder *et al.* 1974; Ebeling and Laur 1985; Bodkin 1988; Levin 1991) and are consequently tightly linked to the success of recruitment and stock stability of many fish species. The structural complexity of macroalgae extends benthic habitat upward into the water column (Ebeling and Laur 1985; Bodkin 1986, 1988) providing refuges for juvenile fishes from larger piscivores (Anderson 1984; Ebeling and Laur 1985; Carr 1994) as well as habitats and increased attachment surface for prey species (Abele 1974; Hicks 1980; Holbrook and Schmitt 1984; Bodkin 1988). Holdfasts also are structurally complex and contain characteristic aggregations of invertebrates (Ghelardi 1971).

1.2. - KELP BED DYNAMICS

Kelp forests are very susceptible to both biotic and abiotic factors and the overall dynamics of their communities are structured by a complex interaction between large-scale oceanographic and local-scale biological processes (North 1971; Dayton *et al.* 1984; Ebeling *et al.* 1985; Foster and Schiel 1985; Dayton *et al.* 1998). Large-scale studies have demonstrated the importance of "bottom-up" processes on kelp forest community dynamics (Dayton *et al.* 1992; Tegner *et al.* 1996; Dayton *et al.* 1999). Several studies (mostly on *Macrocystis pyrifera*) report salinity, light intensity, and temperature to be major physical factors influencing kelp distribution, growth, and survival (North 1979; Lüning 1980; Dean and Jacobsen 1984; Deysher and Dean 1984; Reed and Foster 1984; Dayton 1985; Foster and Schiel 1985; Deysher and Dean 1986a, b; Dayton *et al.* 1999). Other critical physical factors include turbidity, wave exposure, substrate character, and smothering by sediments (Devinny and Volse 1978; Gerard and Mann 1979; Dean and Jacobsen 1984; Ebeling *et al.* 1985; Dayton *et al.* 1992, 1999; Tegner *et al.* 1995; Hurd 2000). Nutrient concentrations, especially nitrogen availability, also play an important role in algal growth and development (Dean and Deysher 1983; Amsler and Neushul 1990; Kopczak 1994; Dayton *et al.* 1999; Kinlan *et al.* 2003). Several studies also have emphasized the importance of numerous biological factors on the regulation of kelp forest communities; factors include competition for space with other plants and animals, self-shading and shading by overstory plants, fouling, diseases, and grazing (North 1971; Dayton 1975; Dayton *et al.* 1984; Dayton and Tegner 1984; Reed and Foster 1984; Denley and Dayton 1985; Foster and Schiel 1985; Harrold and Reed 1985; Dean *et al.* 1989; Reed 1990; North 1991). Abiotic and biotic factors may act independently or in combination and may change with seasons and location. There are probably additional, unknown, factors that complicate kelp bed dynamics that are yet to be identified.

1.3. – SELECTED PHYSICAL PARAMETERS

1.3.1. - Salinity

The term "salinity barrier" is often used to point out the major role salinity plays as an ecological factor in freshwater and marine vegetation distribution (Gessner and Schramm 1971). Although some macroalgae can be found in either habitat, kelps are restricted to marine waters. Several studies have shown that in estuaries the number of species, particularly red and brown algae, rapidly declines inland in inverse proportion to salinity. Few algae are able to osmoregulate their water and mineral contents to survive at low salinities and the occurrence, even of euryhaline species, eventually decreases at the head of estuaries (Scagel 1961; Druehl 1981; Dawson and Foster 1982; Nybakken 1997).

Salinity influences seawater density and electrical conductivity. Because salinity is related to the concentration of ions and osmotic pressure of seawater, it can have dramatic implications on algal physiology (for review see, Gessner and Schramm 1971; Lobban and Harrison 1997). Variation in salinity may be lethal to kelp and other algae as it disrupts the osmotic potential of the cells, causing movement of fluid along water-potential gradients and the flow of ions along electrochemical gradients (Gessner and Schramm 1971; Young *et al.* 1987a). Salinity variations cause changes in ionic composition and metabolite concentrations in cells (Reed *et al.* 1980a, b; Russell 1987; Young *et al.* 1987a, b) as well as the chemical composition of algal thalli (Munda 1961). Kelp gametes and spores are particularly vulnerable to changes in volume and water content due to changes in salinity because they lack cell walls (Russell 1987).

The effects of salinity on algal growth, respiration, and photosynthesis have been studied on several occasions (Norton and South 1969; Kjeldsen and Phinney 1971; Yarish *et al.* 1979a, b; Coudret *et al.* 1983; Greenway and Munns 1983; Gerard *et al.* 1987; Wiencke and Davenport 1987; Herbst and Bradley 1989; Herbst and Castenholz 1994; Conitz *et al.* 2001; Munns 2002). Results and conclusions from these studies have varied greatly depending on species, experimental settings, and acclimation length, suggesting that there is no simple relationship or effect of salinity on seaweed health and fitness. Several studies suggest that salinity levels different from that of the water in which an individual grew may have a negative impact on seaweed growth and development, and that there are optimum salinities for growth, respiration and photosynthesis (Munda 1961; den Hartog 1971; Russell and Bolton 1975; Reed and Russell 1979; Yarish *et al.* 1979a, b; Young *et al.* 1987b). Different populations of the same species may respond differently to salinity stress as a result of phenotypic plasticity (den Hartog 1971; Geesink 1973; Gerard and Mann 1979) or genotypic variations (Russell and Bolton 1975; Bolton 1979; Reed and Russell 1979; Gerard *et al.* 1987; Young *et al.* 1987b), or a combination of both processes (Yarish *et al.* 1979b).

1.3.2. – Light and turbidity

Light plays an important role in structuring seaweed communities. It influences benthic algal distribution both spatially and temporally by affecting growth, production, recruitment, and development (Vadas 1972; Kain *et al.* 1975; Lüning 1981a; Neushul 1981; Dean and Deysher 1983; Dean and Jacobsen

1984; Gerard 1984; Deysher and Dean 1986a; Graham 1996; Han and Kain 1996; Kinlan *et al.* 2003). Both light quality and quantity affect algal rates of metabolic reactions and photosynthetic responses. Minimum light levels are required for survival and growth while excessive irradiance can be fatal; photosynthesis is characterized by compensating and saturating light levels, and critical photoperiod and quantum dose are necessary for photomorphogenic processes (Lüning 1981a, b). The euphotic limit for most kelp is approximately 1% of the light reaching the surface (Lüning and Dring 1979; Lüning 1981a, b). The saturating quantum irradiance for photosynthesis and growth of subtidal laminarians has been measured at approximately 200 $\mu mol/m^2/s$ and 30 to 100 $\mu mol/m^2/s$, respectively (Clendenning 1971b; Lüning 1981b). Light requirements vary depending on the life stage of the plant (Lüning 1981b; Dean and Jacobsen 1984; Deysher and Dean 1984; Foster and Schiel 1985; Deysher and Dean 1986b; Han and Kain 1996) and similar to salinity, different populations of the same species may be genetically adapted or phenotypically acclimated to different light regimes (Kain 1965; Lüning 1981a; Gerard *et al.* 1987; Gerard 1990; Tegner *et al.* 1996).

The amount of light available throughout the water column depends on factors such as surface irradiance, daylength, and water clarity. Sunlight spectrum is modified in intensity and quality as it penetrates the water column due to absorption and scattering. Turbidity refers to the degree to which light traveling through the water is scattered and absorbed by the suspended organic and inorganic particles, such as clay, silt, plankton, and dissolved colored organic compounds. Higher attenuation occurs for longer electromagnetic wavelengths causing red and infra-red radiations to be predominantly absorbed and blue light to penetrate deeper. In clear water, the change in spectral composition is from primarily yellow at the surface to blue green at depth whereas in turbid coastal waters containing high concentrations of dissolved organic material blue light is strongly absorbed and yellow-colored water results (Jerlov 1976; Drew 1983). The distribution of several algae has been related to turbidity levels (Kain 1965; Widdowson 1965; Drew and Jupp 1976; Edwards 1980).

1.3.3. - Temperature

Temperature influences all life processes as it has a fundamental effect on proteins, enzymes, nucleic acids, cell membranes, and on the rate of biochemical reactions (Kinne 1971). As a result of enzyme sensitivity, photosynthesis, respiration, and growth are processes strongly influenced by temperature. Temperature also has been observed to affect macroalgal fertility and nutrient absorption (Lüning and Neushul 1978; Bolton and Lüning 1982; Bolton and Levitt 1985; Gerard *et al.* 1987). Many *in-situ* studies on kelp forest dynamics have reported strong relationships between temperature and macroalgal development, health, and survival (Dean and Jacobsen 1984; Deysher and Dean 1986a; Dayton *et al.* 1992, 1999; Tegner *et al.* 1996). Experiments relating seaweed temperature tolerance in the laboratory to geographical distribution limits have been conducted for many years (Lüning 1984; van den

Hoek 1987; Lüning and Freshwater 1988; Cambridge *et al.* 1990a, b; tom Dieck 1993). Temperature tolerance in plants depends on several criteria, including absolute intensity of temperature extremes, exposure duration to extreme temperatures, rate at which changes occur, and developmental stage of the plant (Kinne 1971). Several studies also have shown that temperature has an interactive effect with other physical factors such as salinity (Druehl 1971; Kjeldsen and Phinney 1971; Gerard *et al.* 1987) and light (Lüning and Neushul 1978; Kain 1979; Lüning 1980; Dean and Deysher 1983; Drew 1983; Dean and Jacobsen 1984).

1.4. - *NEREOCYSTIS LUETKEANA*

Nereocystis luetkeana (Mertens *f.*) Postels *et* Ruprecht, hereafter *Nereocystis*, is the dominant surface-canopy forming kelp in Kachemak Bay. This brown macroalga is found along the Eastern Pacific coast from central California to Umnak Island in the Eastern Aleutians (Druehl 1969; Druehl 1971; Miller and Estes 1989). *Nereocystis* is considered an annual because it produces only one stipe during its lifetime and cannot grow new tissue once the upper stipe is destroyed (Nicholson 1970). Because some individuals that mature late survive until the following spring, *Nereocystis* may be regarded as a facultative biennial (Foreman 1970). As such, *Nereocystis* forests are more sensitive to massive physical and biological disturbances than perennial kelp forests (Dayton 1985). The life history of *Nereocystis* is characterized by an alternation of heteromorphic generations (Figure 1.1). The microscopic stage consists of sexual, gamete-producing haploid male and female gametophytes whereas the macroscopic stage consists of conspicuous spore-producing diploid sporophytes (Dawson and Foster 1982).

Nereocystis has a unique spore release mechanism coupling spore release and the abscission of the ripe sori, with most spores being released within one hour of sorus abscission (Nicholson 1970; Amsler and Neushul 1989a). The environmental parameters that trigger spore release of many macroalgae are only partially understood but it has been observed that *Nereocystis* synchronizes its sori release in batches, with up to 80% of the sori monitored in laboratory culture being released within two hours before and four hours after sunrise (Amsler and Neushul 1989a, b). The timing of spore release at dawn is thought to allow the photosynthetic spores to take advantage of solar irradiance immediately after being released and to maximize their carbon reserves, therefore extending their planktonic survival and increasing their germination success (Amsler 1988; Amsler and Neushul 1991). Young *Nereocystis* sporophytes usually appear on the sea floor in early spring in British Columbia (Duncan 1973; Wheeler *et al.* 1984); the earliest identifiable young sporophyte was observed on April 26 (2001) in Kachemak Bay, Alaska (personal observation). The majority of *Nereocystis* plants are reproductive by late June in Puget Sound, Washington (Maxell and Miller 1996); while fertile sporophytes were first observed between late June and late July in different locations in Kachemak Bay (personal observation). *Nereocystis* reach their maximum canopy

cover between May and July in Puget Sound, Washington (Maxell and Miller 1996), California, and British Columbia (Wheeler *et al.* 1984), and in late July and early August in Prince William Sound, Alaska (Stephen Jewett, University of Alaska Fairbanks, personal communication). In Kachemak Bay, *Nereocystis* canopy cover reaches the surface by early August (personal observation). Within Kachemak Bay, the phenology of *Nereocystis* appears somewhat delayed in the inner bay (Halibut Cove) compared to the outer bay (Port Graham) (personal observation).

1.5 - KACHEMAK BAY

The study area was Kachemak Bay, located in the Lower Cook Inlet, Alaska (Figure 1.2). Kachemak Bay is the nation's largest National Estuarine Research Reserve and is approximately 50 km wide at the entrance, 80 km long, and covers an area of 1480 km^2 with 540 km of shoreline. Kachemak Bay displays characteristic salinity and turbidity gradients along its axis. Salinity decreases and turbidity increases from the entrance to the head of the bay as inputs of freshwater and sediments from rivers and glaciers become more abundant. As several glaciers from the Harding Icefield feed into the bay, glacial silts are a major cause of turbidity in Kachemak Bay and the Cook Inlet (Trasky *et al.* 1977; Lees *et al.* 1980). Hydrographic data collected by two YSI ocean sensors (deployed by Kachemak Bay Research Reserve, KBRR), located 1 m above the seafloor, on the inside of the Homer Spit and at the ferry dock in Seldovia Bay show a salinity and turbidity gradient between the inner and outer bay. The salinity at the Homer spit reaches 34‰ in the winter but drops to 20‰ in the summer whereas the salinity in Seldovia remains close to 34‰ throughout the year. The turbidity is around 14-15 NTU's (Nephlometric turbidity units) in Homer but only 2-4 NTU's in Seldovia. Due to the stratification in the summer, surface salinities are even lower than the bottom salinities and may drop to 10‰ in Homer (Carl Schoch, KBRR, personal communication). Kachemak Bay is an estuary during a few summer months but is predominantly a marine system for most of the year. Early in the year, when the run-offs are frozen, the salinity gradient between Port Graham (outer bay) and Halibut Cove (inner bay) is limited and the bay is a fairly homogeneous and well-mixed marine system. In late spring, the rivers start flowing and stratification increases (mostly at the head of the bay), after which the system slowly switches from marine to estuarine. The most prominent changes and strongest stratification are observed in Halibut Cove (in the inner bay) (Carl Schoch, KBRR, personal communication). Kachemak Bay is considered a positive estuary because precipitation and run-off exceed evaporation, although the amount of freshwater input is reduced in the winter and spring when run-offs are frozen (Trasky *et al.* 1977). Kachemak Bay is, however, an unusual estuary because the volume of seawater entering the bay daily is five orders of magnitude greater than the daily amount of freshwater (approximately $14 \times 10^9 m^3$ versus $7 \times 10^5 m^3$ respectively). Moreover, the watershed is less than twice the size of the bay area (the typical ratio of watershed to estuary area is greater than 10:1) (Carl Schoch,

KBRR, personal communication). Kachemak Bay also has an extreme tidal range of 8.5 m, with a mean of 4.7 m (only second to the Bay of Fundy). The massive semi-diurnal tides create strong oscillatory currents that have an important influence on the overall water circulation pattern of Kachemak Bay. Despite the tremendous mixing effects of the tidal currents, in the summer, the water column remains well stratified, especially in the inner bay. This alternation between upwelling of nutrients with the input of new oceanic water twice a day and strong stratification may result in high primary production.

The overall water circulation in Kachemak Bay is generated by an input of upwelled clear oceanic water from the Gulf of Alaska at the southwest entrance of the bay and an outflow of fresher and more turbid water on the north side into Cook Inlet. The Homer spit, a natural projection that extends 4 km into the bay from the northern shore, splits Kachemak Bay into an inner and outer bay system. A cyclonic gyre resides on each side of the spit (Figure 1.3) (Burbank 1977). A drift card study corroborated Burbank's results and indicated the presence of a very strong surface outflow from the inner to the outer bay and very little surface flow from the outer into the inner bay (Schoch 2001). This general circulation pattern is a summer feature with the outflow from the inner to outer bay weakening during the winter (Carl Schoch, KBRR, personal communication).

In Kachemak Bay, aerial surveys have recorded approximately 31 km^2 of *Nereocystis* forests and have shown a gradient in bed size and density along the bay axis (Schoch 2001) (Figure 1.2). The beds are dense at the mouth of the bay where clear oceanic water enters. Only solitary stands are observed in the inner bay and no kelp was found at the head of the bay past Halibut Cove, although suitable substrate is available. The general kelp bed distribution is similar to 20 years ago, when it was suggested that the absence of well-developed surface canopies on the northern shelf of Kachemak Bay and the west side of Lower Cook Inlet was related to the presence of turbid waters (Lees *et al.* 1980).

1.6. –STUDY OBJECTIVES

This study investigated the influence of an estuarine versus marine environmental regime on the development and growth of *Nereocystis luetkeana* in Kachemak Bay, Alaska. Since *Nereocystis* has a complex life cycle both the macroscopic and microscopic life stages were examined. The effect of herbivory pressure on the survival of *Nereocystis* at a local scale was also investigated. This project focused on three objectives:

1- The effects of salinity and turbidity on the *in-situ* survival and growth rates of *Nereocystis* sporophytes.

2- The effects of salinity and light intensity on the development of *Nereocystis* spores.

3- The effects of grazing on the survival of *Nereocystis* sporophytes.

While the effects of salinity and turbidity as well as grazing on marine algae have been assessed in a number of studies, the effects on *Nereocystis* have received little attention. The role of salinity and light intensity on the growth of *Nereocystis* sporophytes was investigated by performing reciprocal transplants of juveniles among three beds along a salinity and turbidity gradient and by regularly measuring their growth rates throughout the summer. The effects of salinity and light intensity on the development of *Nereocystis* spores were studied in the laboratory. The sinking tendency, settlement success, germination success, and germ tube length of spores were monitored under three salinity and four light intensity levels. The effect of grazing on the survival of *Nereocystis* sporophytes was studied by monitoring the survival of the transplants at the three study sites and by recording the timing of recruitment and abundance of a major kelp herbivore, the gastropod *Lacuna vincta*. The three major areas of investigation (*in-situ* experiment, laboratory experiment, and grazing study) are presented in separate chapters.

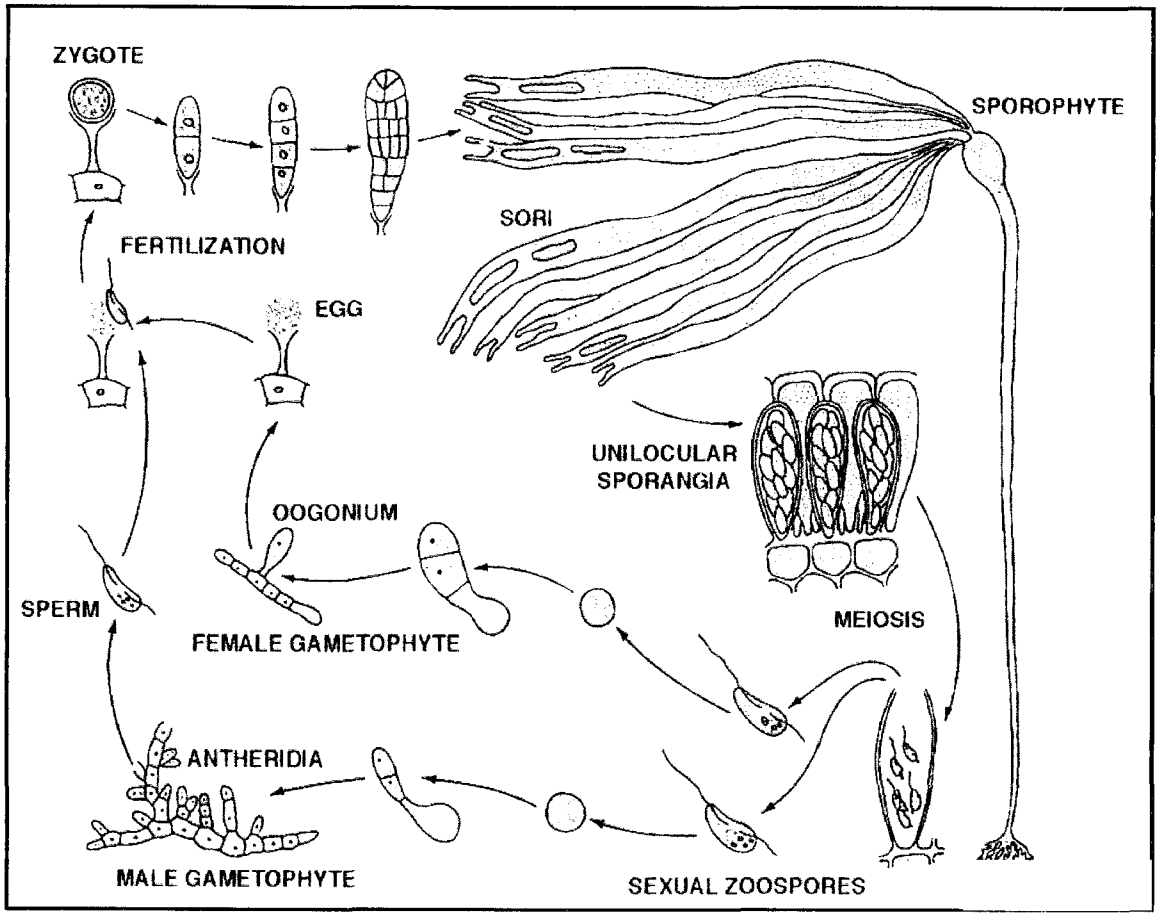

Figure 1.1: Life cycle of *Nereocystis luetkeana* (Diagram from Scagel *et al.* 1982).

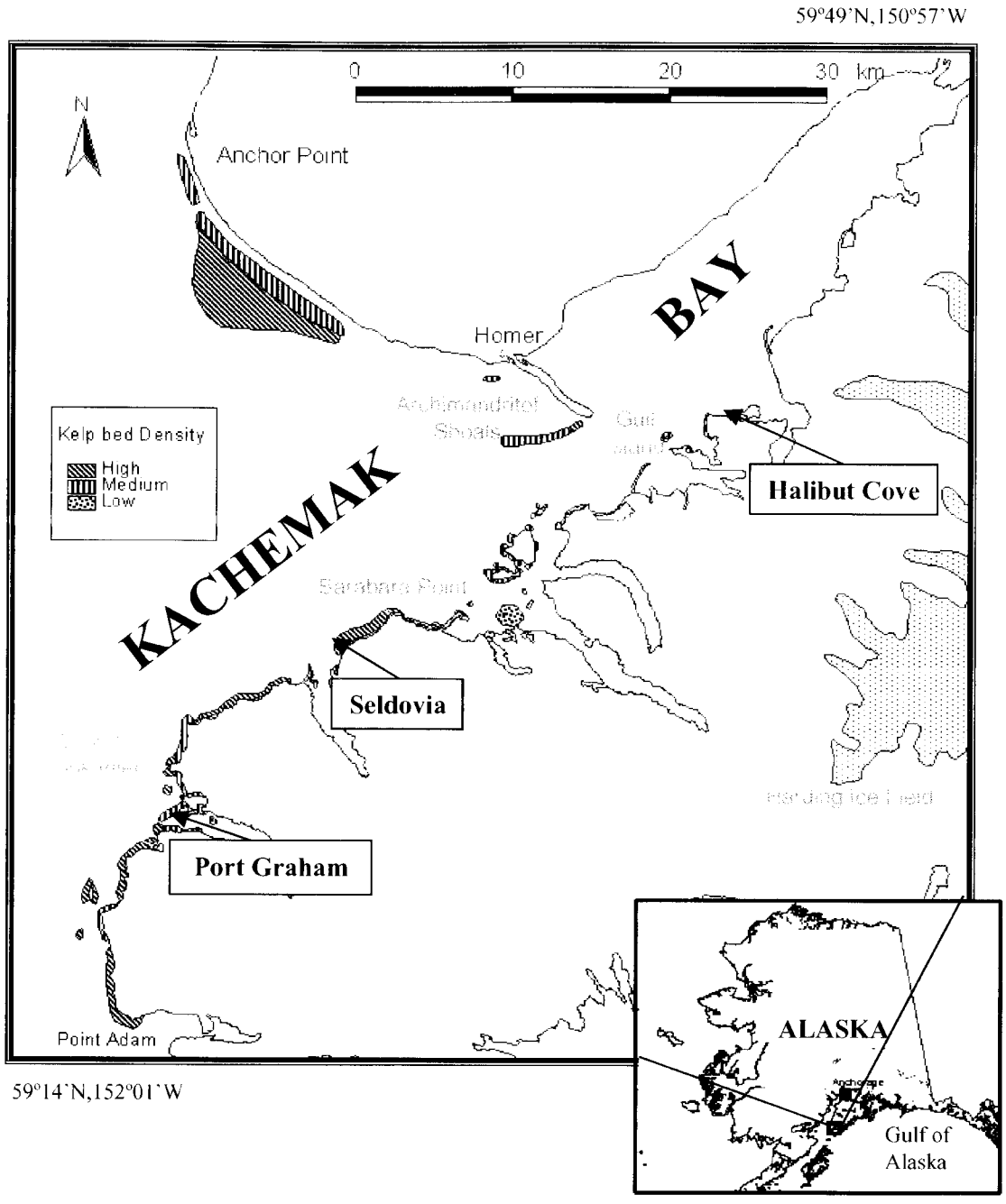

Figure 1.2: Map of the study sites (Port Graham, Seldovia, and Halibut Cove) and the kelp beds of Kachemak Bay. The location and size of the kelp beds were estimated from an aerial survey conducted by the Kachemak Bay Research Reserve in August 2000. Kachemak Bay is located in the Lower Cook Inlet in the Gulf of Alaska.

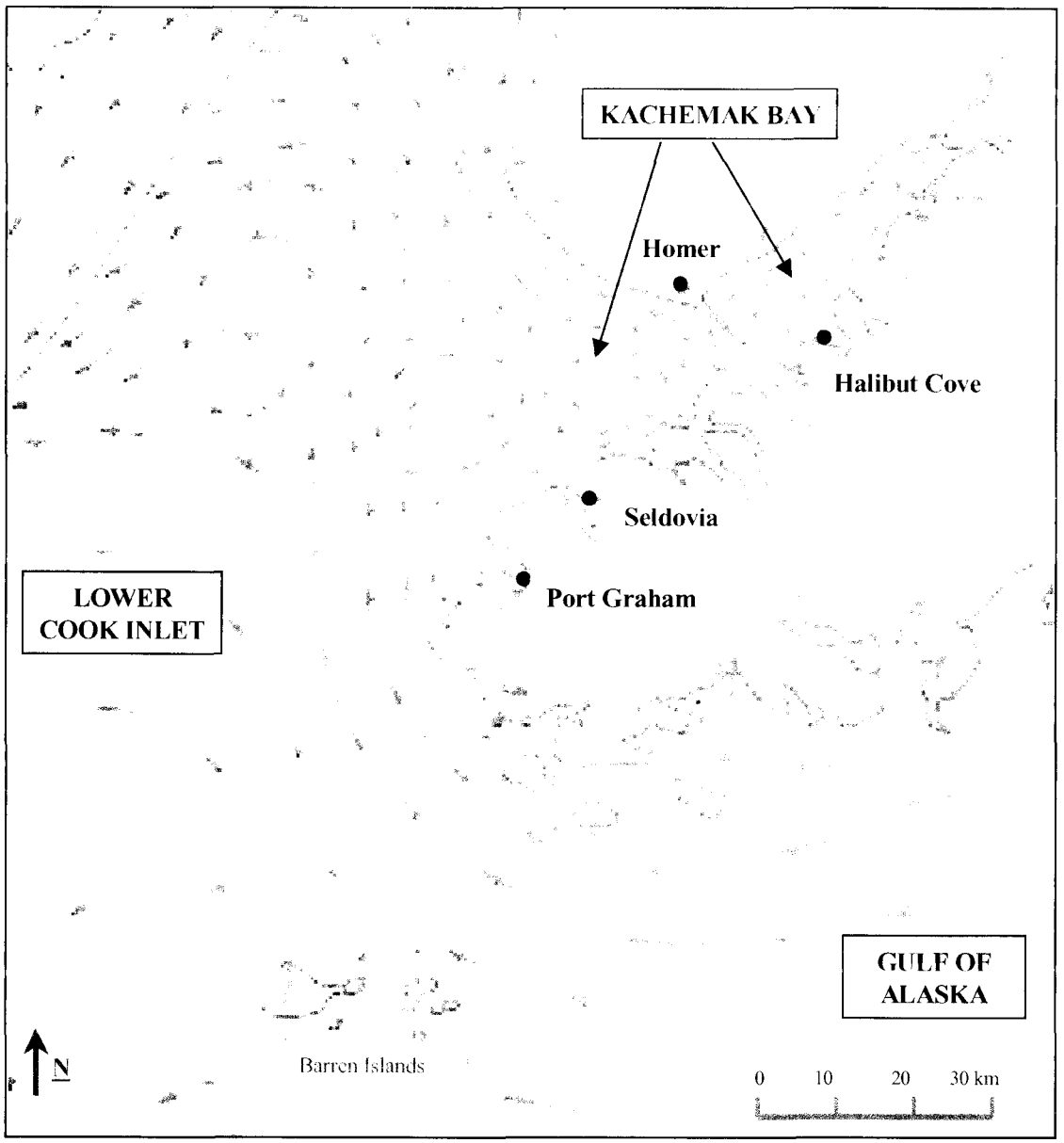

Figure 1.3: Surface circulation patterns in Lower Cook Inlet and Kachemak Bay (Diagram modified from Burbank 1977).

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CHAPTER 2

ROLE OF SALINITY AND TURBIDITY ON THE GROWTH AND SURVIVAL OF *NEREOCYSTIS LUETKEANA* IN KACHEMAK BAY, ALASKA

2.1. - INTRODUCTION

Fluctuations in structure, composition, abundance, and health of kelp forests are thought to result from a complex combination of physical, chemical, and biological factors (North 1971; Dayton *et al.* 1984, 1998; Ebeling and Laur 1985; Foster and Schiel 1985). "Bottom-up" processes are critical to kelp forest community dynamics (Dayton *et al.* 1992, 1999; Tegner *et al.* 1996) with salinity, light attenuation, turbidity, and temperature amongst the major physical factors influencing kelp growth and survival rates (Druehl 1978; North 1979; Dean and Jacobsen 1984; Deysher and Dean 1984; Reed and Foster 1984; Dayton 1985; Deysher and Dean 1986; Wheeler and Druehl 1986; Dayton *et al.* 1992, 1998, 1999).

The effects of salinity on algal growth, respiration, and photosynthetic responses have been studied on several occasions (Norton and South 1969; Kjeldsen and Phinney 1971; Bolton 1979; Yarish *et al.* 1979a, b; Norton *et al.* 1981; Coudret *et al.* 1983; Gerard *et al.* 1987; Wiencke and Davenport 1987; Herbst and Bradley 1989; Herbst and Castenholz 1994), but results varied greatly depending on species, experimental settings, and acclimation length. Several studies suggested that salinity levels different than that of the water in which an individual grew may have a negative impact on algal growth and development (den Hartog 1971; Coudret *et al.* 1983; Greenway and Munns 1983; Gerard *et al.* 1987; Herbst and Bradley 1989; Herbst and Castenholz 1994). The growth rate of the red alga *Agraothamnion chadefaudii* was negatively affected by reduced salinity (Coudret *et al.* 1983) whereas that of the filamentous green *Ctenedadus circinnatus* was reduced under high salinity (Herbst and Castenholz 1994). In estuaries, the number of species rapidly declined inland in inverse proportion to salinity (particularly red and brown algae). Few algae are able to adjust their water and mineral contents to survive at low salinities; even the occurrence of euryhaline algae eventually decreased towards the head of estuaries (Scagel 1961; Gessner and Schramm 1971; Druehl 1981; Dawson and Foster 1982). Kelps, including *Nereocystis luetkeana*, are restricted to marine habitats and are rarely found in areas of reduced salinities, such as estuaries (Druehl 1981; Dayton 1985; Nybakken 1997).

Light is a primary factor structuring patterns of benthic algal communities by influencing species composition, abundance, and primary production (Lüning 1981a, b; Drew 1983; Dean and Jacobsen 1984; Gerard 1984; Foster and Schiel 1985; Deysher and Dean 1986; Gerard 1990; Han and Kain 1996). Light quality and quantity affect photosynthetic responses and metabolic patterns. The minimal light irradiance required for most kelp is approximately 1% of the light reaching the surface (Lüning 1979, 1981b). For

instance, the minimal irradiance required for growth of juvenile sporophytes of North Pacific *Macrocystis pyrifera* was measured at 0.4 - 0.7 E/m^2/d (ca. 4.6 - 8.1 µmol/m^2/s) (Dean and Jacobsen 1984). However, light requirements vary depending on the algal life cycle stage (Kain 1965; Foster and Schiel 1985; Gerard 1990; Tegner *et al.* 1996). Adult kelps that have reached the surface appeared to be less sensitive to reduced subsurface light than young sporophytes (Lobban 1978).

The growth of *Nereocystis* thalli is strongly influenced by light quantity and quality (Duncan 1973; Duncan and Foreman 1980). The elongation rate of both blades and stipe of mature *Nereocystis* sporophytes decreased when exposed to low irradiance (Duncan 1973). When *Nereocystis* sporophytes reach the surface, their stipe stopped growing whereas their blades elongated faster and their biomass increased (Nicholson 1970). Also, the plants display a rapid elongation burst right before the stipe ceased to grow, presumably in response to a low ratio of red:far-red wavelengths in the upper water column (Duncan 1973). The pattern of stipe elongation in *Nereocystis* was not solely regulated by photosynthesis and may be a 'phytochrome-like response" (Duncan 1973; Duncan and Foreman 1980).

The amount of light available to photosynthetic organisms throughout the water column depends on factors such as depth, surface irradiance, day length, and flatness of the surface; shading by the overstory can also reduce light irradiance reaching the seafloor (Jerlov 1976; Dayton *et al.* 1984; Reed and Foster 1984; Kennelly 1988; Duggins *et al.* 1990; Schroeter *et al.* 1995; Graham *et al.* 1997). High turbidity levels, a common feature of estuaries, interfere with the penetration of sunlight through the water column thus limiting the amount of irradiance available to gametophytes and young sporophytes (Kain *et al.* 1975; Drew and Jupp 1976). Turbidity levels dictate algal depth occurrence and distribution along estuary axes (Edwards 1980).

Because of the effects of low salinity, low light levels, and high turbidity associated with estuarine environments, kelps usually do not flourish in estuaries. In Kachemak Bay, the density and presence of *Nereocystis luetkeana* kelp beds decrease from the mouth toward the head of the bay. The hydrographic conditions change from oceanic (high salinity and low turbidity) to estuarine (low salinity and higher turbidity) along that axis. The *Nereocystis* kelp bed found furthest inside the bay is located in Halibut Cove, which is approximately 50 km away from the entrance of the bay and 25 km from the head of the bay. No *Nereocystis* kelp stands are found inland past that point. These observations led to the idea that the distribution of *Nereocystis* kelp beds within Kachemak Bay may be influenced by a detrimental estuarine effect on the growth and successful establishment of *Nereocystis*. The purpose of this study was to investigate the role of those hydrographic factors on growth and survival of *Nereocystis* by performing reciprocal transplants of juveniles among three beds along a salinity and turbidity gradient in Kachemak Bay.

2.2.- METHODS

2.2.1. - Sites description

This study was conducted in Kachemak Bay, in Lower Cook Inlet, Alaska. Three kelp beds were chosen along the bay axis so that each was characterized by different salinities and turbidities (Figure 1.2). Port Graham (N 59° 22.270', W 151° 53.728'), the outer bay site, is located toward the open sea and is characterized by clear oceanic water (high salinity and low turbidity). Halibut Cove (N 59° 35.866', W 151° 16.141'), the inner bay site, is located toward the head of the bay and is characterized by more brackish water (lower salinity and higher turbidity). Seldovia (N 59° 28.074', W 151° 42.636') is located between the two other sites, 30 km southwest from Halibut Cove and 20 km northeast form Port Graham. The selected sites were as similar as possible regarding wave exposure, wave energy regimes, and substrate. All sites were in 8 m water depth. In Kachemak Bay, the tides have a mean amplitude of 4.7 m, with an extreme of 8.5 m, and create strong oscillatory currents throughout the bay.

Nereocystis luetkeana is the major canopy-forming kelp in Kachemak Bay, Alaska. The sporophytes of *Nereocystis* appear on the seafloor by mid-Spring, become reproductive by mid-July, and reach their maximum canopy in the fall. The timing of these events is a little earlier for beds at the entrance of the bay, and somewhat later for beds in the inner bay (personal observations).

2.2.2. - Hydrographic Measurements

2.2.2.1. - Salinity and turbidity profiles

Water samples were collected in 250-mL water bottles from three different depths at each site on three occasions during Summer 2001 (July 23, July 30, and August 6). Scuba was used to collect samples 50 cm above the seafloor and in mid-water column whereas surface samples (approximately 20 cm below surface) were collected from a skiff. Salinity and turbidity were later analyzed in the laboratory using a multiparamter water quality monitor (YSI, model 6600) and an NTU turbidimeter (LaMotte, model 2020), respectively. A one-way ANOVA followed by a Tukey test was performed to determine significant differences in salinity and turbidity among sites (SAS Institute Inc., 1999).

2.2.2.2. – Light intensity profiles

On three occasions during the summer (July 30, August 6, and August 14, 2001), light intensity profiles were shaped at each site by measuring light intensity in the visible spectrum (Photosynthetically Active Radiation, PAR) at different depths (0, 1, 2, 3, 4, 5, and 8 m) using a spherical PAR quantum sensor (Li-Cor, model LI-193SA). Because the measurements were not simultaneous at all three sites, the incoming solar radiation varied due to changing cloud cover and sun angle; therefore, the percentage of light available at different depths is in reference to the solar radiation measured right below the surface

instead of absolute quantum photons. A one-way ANOVA followed by a Tukey test was performed to determine potential significant differences in light attenuation among sites (SAS Institute Inc., 1999).

2.2.2.3. – Continuous light and temperature measurements

Light intensity was used as a proxy for turbidity because there is a strong correlation between turbidity and light availability throughout the water column (Jerlov 1968, 1976; Drew 1983). It was assumed that, on average, factors such as amount and angle of incoming solar radiation and day length, which are known to alter light levels, were similar at all sites. Although cloud cover and surface roughness are more susceptible to variations among sites at any given time, overall, differences in the amount of light reaching the bottom at each site was chiefly attributed to varying turbidity levels in the water column. It also was logistically easier to collect continuous light data instead of continuous turbidity data. A light intensity sensor (Onset, Model HOBO) was placed at 8 m MLLW (Mean Lower Low Water) at each site and measurements (in log lumens/m^2) were made every hour from July 8 until August 16, 2001. A total of 960 hourly readings were collected. Daily means and daily maxima were calculated and compared between sites. The variation in light intensity daily means and the averaged daily mean values were compared between sites using a one-way ANOVA followed by a Tukey test (SAS Institute Inc., 1999).

Continuous temperature measurements also were collected at a depth of 8 m MLLW at each site for the duration of the *in-situ* experiment. The measurements were recorded every ten or fifteen minutes for a total of forty days with a temperature data logger (Onset, Model Stow Away IS Temperature). The temperature sensor in Halibut Cove failed to record data for 48 hours between July 10 and12 and unexpectedly changed sampling intervals from ten to fifteen minutes. The difference in mean daily temperatures and the mean daily variation between minimum and maximum daily values were compared between sites using a one-way ANOVA followed by a Tukey test (SAS Institute Inc., 1999).

In contrast with the occasional salinity, turbidity and PAR measurements, light and temperature sensors collected data at intervals less than half the tide cycle. Consequently, daily averages are thought to be unbiased by tidal activities. The light and temperature sensors placed on the ground line (see below) at each study site gathered information on the amplitude and frequency of variations in those two parameters throughout the study.

2.2.3. - Transplants

Reciprocal transplants were performed amongst three study sites to determine potential differences in growth and survival of *Nereocystis* transplants depending on their site of origin. A total of 36 *Nereocystis* juveniles were collected from each site, out of which twelve were transplanted to each study site. For example, of the 36 individuals collected from Port Graham, 12 were transplanted to Port Graham, 12 to Seldovia, and 12 to Halibut Cove. Prior to kelp transplantation, four ground lines along the 8 m

MLLW contour were placed at each site (Figure 2.1). The ground lines were approximately 8 m long and haphazardly spaced 5 to 10 m apart. Each end of the line was securely attached to heavy weights. Three plants from each of the three origins were haphazardly attached on each of the four ground lines. The transplants were approximately 50 cm apart, which is similar to natural densities (personal observation; Lees *et al.* 1980). Port Graham and Seldovia kelp beds were extensive and dense, whereas the kelp bed in Halibut Cove was confined to a small pinnacle (approximately 100 x 25 m) at the entrance of the cove (Figure 1.2). The ground lines were located within the kelp beds in Port Graham and Seldovia but were 10-50 m away from the kelp bed edge in Halibut Cove.

Healthy juvenile plants were collected between July 4 and July 7 and most were transplanted on the day of collection; however, because of the great distances between sites, some were kept in coolers with running seawater overnight at the Kasitsna Bay laboratory before being transplanted the next day. The selected transplants had an average stipe length of 48.4 ± 0.9 cm (SE; n = 108); the shortest individual was 30 cm and was collected from Port Graham and transplanted in Halibut Cove and the longest transplant was 76 cm and was collected from Seldovia and transferred to Port Graham. Before taking the transplants underwater, the holdfast of each transplant was carefully weaved through a soft nylon line and secured with cable ties. This technique limited stipe and holdfast abrasion. The nylon line was tied onto a small, labeled float equipped with a gagnion hook (Figure 2.1). The design had several advantages as the small floats provided a standard substrate on which the holdfasts were secured and prevented potential urchin grazing by keeping the ground lines off the seafloor. In addition, this system allowed for minimal handling of the transplants underwater; once ready, the transplants were clipped onto the ground lines using the gagnion hook.

The first transplantation trial of Seldovia and Halibut Cove plants to Port Graham was not successful; those transplants were replaced on the second site visit on July 16. No more problems occurred once transporting techniques were perfected and transplants were protected from heat, sunlight, and desiccation by being transported in sealed coolers filled with seawater. As a consequence, there are only four weeks of measurements for Halibut Cove and Seldovia plants in Port Graham. Although light and temperature conditions varied throughout the summer causing plants transplanted late to grow under hydrographic conditions slightly different than original transplants, data were normalized to "days in water" for statistical comparisons. The amount of time spent in the water seemed more relevant than the transplant date because it allowed comparisons between plants that were transplanted at similar size (ca. 50 cm) and for the same amount of time.

2.2.4. – Growth and survival of *Nereocystis*

Underwater stipe length and survivorship measurements were performed weekly for 6 weeks from July 4 until August 16, 2001 using scuba. Growth measurements were derived from procedures used by

Maxell and Miller (1996). On each sampling event, the stipe total length was assessed by measuring the distance between holdfast and pneumatocyst. The stipe specific growth rate (SGR) was expressed in cm/day and was determined by dividing the number of centimeters gained between two measurements by the number of days elapsed between the two events. The following equation was used:

$$SGR = (L_2 - L_1) / (t_2 - t_1),$$

where $L_1$and L_2 are the original and final stipe lengths, respectively, and $(t_2 - t_1)$ represents the time elapsed in days between observations. Survivorship at each site was expressed as a percentage and was calculated by comparing the number of individuals of each origin alive on different census days to the number of plants initially transplanted. Variance in survivorship at each site was based on the survivorship of transplants from all three origins.

An ANOVA for repeated measures was used to determine differences in stipe lengths, growth rates, and survival rates among sites and origins (SAS Institute Inc., 1999). Because only four weeks of data were available for transplants from Seldovia and Halibut Cove to Port Graham, a statistical analysis was performed for the first four weeks on a model including all transplants and a second analysis was performed for weeks 4, 5, and 6 on a model including all transplants in Seldovia and Halibut Cove, and Port Graham transplants in Port Graham.

Correlations between the SGR of transplants and weekly means in temperature and light intensity at each site were investigated because the data collected by temperature and light sensors at each study site showed that those two factors varied throughout the summer. The correlations were based on the growth rate measured over a certain period and mean temperature and mean light level averaged over that same period for each study site. The correlations between SGR and those two factors were calculated separately for transplants of different origins (the site of transplantation was not taken into consideration).

2.3. - RESULTS

2.3.1. - Hydrographic measurements

2.3.1.1. - Salinity and turbidity profiles

Salinity differences were found between sites (Figure 2.2). Mean salinity for all depths was 26.1 ± 1.5‰ in Halibut Cove (Inner bay site) and was significantly lower than in Seldovia and Port Graham (30.7 ± 0.7‰ and 31.1 ± 0.8‰, respectively) (ANOVA; $df = 2$; $F = 7.07$; $p = 0.0054$; n = 3). Salinities measured at the bottom and in mid-water column were not significantly different between the inner bay and outer bay sites (ANOVA; $df = 2$; $F = 1.34$ and $p = 0.3299$ for bottom salinity; and $F = 1.29$ and $p = 0.3422$ for mid-water; n= 3); however, the surface salinity was significantly lower in Halibut Cove than in Port Graham

and Seldovia (ANOVA; $df = 2$; $F = 7.47$; $p = 0.0235$; n = 3). Although there was no significant depth effect at any of the sites, salinity declined from 29.3 at depth to 22.9‰ at the surface in Halibut Cove. Salinity was more homogeneous through the water column at the two sites closer to the entrance of the bay (Port Graham and Seldovia) varying up the water column from 32.3 to 29.9‰.

Turbidity was higher in Halibut Cove (1.09 ± 0.21 NTU) than in Seldovia (0.33 ± 0.06 NTU) and in Port Graham (0.41 ± 0.07 NTU) (ANOVA; $df = 2$; $F = 9.17$; $p = 0.0018$; n = 3) (Figure 2.3). Although there was no significant difference among depths at any site (ANOVA; $df = 2$; $F = 1.87$; $p = 0.1821$; n= 3), turbidity at the bottom was somewhat higher than in the upper water column due to particle resuspension.

The interpretation of sporadic salinity and turbidity measurements is somewhat complicated because these factors were strongly altered by flowing and ebbing tides, which caused measured values to greatly vary temporally. However, despite strong tidal activities, salinity and turbidity profiles suggested a vertical salinity gradient throughout the water column in Halibut Cove with the depth of the freshwater layer overlying a more oceanic water mass varying with tidal cycle.

2.3.1.2. – Light intensity profiles

Incoming solar radiation measured at the surface during the three sampling dates varied from 2020 to 297 µmol/m^2/s depending on cloud cover and time of the day. Light intensity measured at depth varied between 314 and 40 µmol/m^2/s, 171 and 34 µmol/m^2/s and 59 and 12 µmol/m^2/s in Port Graham, Seldovia and Halibut Cove, respectively. In an effort to normalize light attenuation values, light profiles represent the percentage of PAR available throughout the water column in reference to solar radiation penetrating the surface. Light profiles support the idea that Halibut Cove was more turbid than Port Graham and Seldovia (ANOVA; $df = 2$; $F = 7.37$; $p = 0.0018$; n = 3) (Figure 2.4). At a depth of 5 m, 28.0 ± 3.6% of incoming solar radiation was available in Halibut Cove versus 38.8 ± 4.3% and 45.4 ± 4.0% in Port Graham and Seldovia, respectively (ANOVA; $df = 2$; $F = 4.59$; $p = 0.0618$; n = 3) In Halibut Cove, only 8.7 ± 0.8% of PAR reached the seafloor whereas 17.5 ± 2.7% and 19.9 ± 1.5% of subsurface light intensity was available at depth in Seldovia and Port Graham, respectively (ANOVA; $df = 2$; $F = 10.47$; $p = 0.0111$; n = 3).

2.3.1.3. – Continuous light and temperature measurements

Fluctuations in daily mean light levels between sites appeared synchronous among sites, suggesting the overall pattern was dictated by similar variations in cloud cover (Figure 2.5). However, on average the light levels measured in Halibut Cove were lower than in Port Graham and Seldovia suggesting that water turbidity was higher in Halibut Cove than in Port Graham. Based on 960 hourly measurements at each site, Seldovia received approximately 92% and Halibut Cove 74% of the light intensity measured in Port Graham. The summer daily means were significantly greater in Port Graham than in Halibut Cove (ANOVA; $df = 2$; $F = 9.42$; $p = 0.0002$; n = 40) and on average, 0.81 ± 0.03 log lumen/m^2 were measured

hourly in Port Graham versus 0.74 ± 0.04 log lumen/m^2 in Seldovia and 0.68 ± 0.3 log lumen/m^2 in Halibut Cove. The same pattern was observed when comparing averaged daily maxima (ANOVA; $df = 2$; $F = 7.19$; $p = 0.001$; n = 40).

The most extreme summer temperatures occurred in Halibut Cove with a minimum of 7.6° C on July 18 and a maximum of 12.8° C on July 20 (Figure 2.6). The minimum temperatures were 8.5° C and 8.4° C and the maximum temperatures were 11.8° C and 12.0° C for Port Graham and Seldovia, respectively. The daily mean temperatures (9.8 ± 0.1° C in Port Graham and 9.7 ± 0.1° C in both Seldovia and Halibut Cove) were not significantly different (ANOVA; $df = 2$; $F = 0.27$; $p = 0.7606$; n = 40 for Port Graham and Seldovia and n = 38 for Halibut Cove). In contrast, Halibut Cove had lower daily minima (ANOVA; $df = 2$; $F = 37.55$; $p < 0.0001$; n = 40 or 38) and higher daily maxima (ANOVA; $df = 2$; $F = 49.13$; $p < 0.0001$; n = 40 or 38) than Port Graham or Seldovia. The averaged daily minima were 9.5 ± 0.1° C, 9.6 ± 0.1° C and 8.4 ± 0.1° C, and the averaged daily maxima were 10.0 ± 0.1° C, 10.0 ± 0.1° C and 11.7 ± 0.1° C for Port Graham, Seldovia and Halibut Cove, respectively. As a result, the daily variation was greater in Halibut Cove than at the other two sites (ANOVA; $df = 2$; $F = 346.06$; $p < 0.0001$; n = 40 or 38). On average, the temperature varied daily by 3.2 ± 0.1° C in Halibut Cove but only by 0.5 ± 0.04° C at both other sites. There was a lag in temperature patterns of up to two days between Port Graham and Seldovia versus four to five days between Port Graham and Halibut Cove.

2.3.2. – Growth and survival of *Nereocystis*

2.3.2.1 – Specific growth rate

Specific growth rate (SGR) for all plants increased as plants grew taller (Figure 2.7 a, b, c). During the first two weeks, SGR ranged from 0.3 ± 0.1 cm/day for Port Graham transplants in Port Graham up to 2.3 ± 0.4 cm/day for Halibut Cove transplants in Halibut Cove. Between week 5 and 6, SGR varied from 2.9 ± 0.9 cm/day for Port Graham transplants in Halibut Cove up to 9.0 ± 1.2 cm/day and 9.0 ± 0.7 cm/day for Seldovia transplants in Seldovia and in Halibut Cove, respectively. The greatest SGR was 14.7 cm/day by an individual Halibut Cove transplant in Halibut Cove. In contrast, for some measurement periods, certain plants did not grow.

In Port Graham, transplants from all origins grew at similar rates (ANOVA; $df = 2$; $p > 0.05$ for weeks 3 and 4). In both Seldovia and Halibut Cove on weeks 2 through 5, Port Graham transplants had a significantly smaller SGR than Seldovia and Halibut Cove plants (ANOVA; $df = 2$; $p < 0.05$). In Halibut Cove on week 6, the SGR of Port Graham transplants reached that of Halibut Cove transplants, and Seldovia transplants started growing at a higher rate than Halibut Cove transplants (ANOVA; $F = 8.23$; $p = 0.0015$). In Seldovia, Seldovia transplants started growing faster than Halibut Cove transplants between week 3 and 4 ($p < 0.05$ for weeks 4, 5 and 6). The SGR of Seldovia and Halibut Cove transplants was not available for weeks 5 and 6 in Port Graham.

Plants, especially those from Halibut Cove, appear programmed to grow at a maximum rate that cannot be exceeded even under putatively optimal growing conditions. In both Halibut Cove and Seldovia, Halibut Cove transplants reached a maximum SGR of approximately 5.5 to 6.0 cm/day between week 4 and 5 and did not increase between week 5 and 6. Seldovia transplants also showed an apparent maximum SGR in Seldovia of approximately 9.0 cm/day, but not in Halibut Cove where their SGR kept increasing from 7.6 ± 0.4 cm/day to 9.0 ± 0.7 cm/day between week 5 and 6. When comparing the SGR of Halibut Cove transplants at all sites, there was no significant difference ($p > 0.05$), although Halibut Cove plants grew slightly slower in Port Graham than in Seldovia and Halibut Cove on week 3 (ANOVA; $df = 2$; $F = 5.08$; $p = 0.0131$). In contrast, Port Graham transplants grew slower when transplanted from an oceanic to an estuarine system (ANOVA; $df = 2$; $F = 5.76$ and $p = 0.0081$ on week 4; $F = 7.37$ and $p = 0.0034$ on week 5). The SGR of Port Graham transplants in Port Graham increased through the summer and reached 6.8 ± 1.5 cm/day on week 6. In Seldovia, the SGR of Port Graham transplants remained below 1.1 cm/day until week 4 and increased by 116% from week 4 to week 5 (from 1.1 ± 0.3 cm/day to 2.4 ± 0.4 cm/day) and by 85% from week 5 to week 6 (from 2.4 ± 0.4 cm/day to 4.4 ± 1.4 cm/day). In Halibut Cove, the increase was delayed until week 5 when it rose to155% (from 1.1 ± 0.4 cm/day on week 5 to 2.9 ± 0.9 cm/day on week 6).

The SGR for transplants of all origins and at all sites responded positively to increasing temperatures ($r = 0.65$) (Figure 2.8). When examining the correlation for transplants of different origins separately, Port Graham transplants seemed to respond more strongly to warmer temperatures ($r = 0.86$) than Seldovia and Halibut Cove transplants ($r = 0.62$ and $r = 0.55$, respectively). The correlation between SGR and light levels was negative ($r = -0.68$) (Figure 2.9); as light levels decreased, SGR of transplants increased. The SGR of transplants from various origins responded more similarly to light levels than they did to temperatures ($r = -0.71$, $r = -0.70$ and $r = -0.77$ for Port Graham, Seldovia and Halibut Cove transplants, respectively).

2.3.2.2 – Survivorship

No obvious effects of estuarine versus oceanic conditions on transplant survivorship were documented in this study. The overall transplant survivorship remained above 83.7 ± 3.8% for the first six weeks of the experiment (Figure 2.10). On August 15, 2001, 87.3 ± 3.7% of the transplants were still alive in Port Graham, 75.3 ± 8.3% in Seldovia, and 88.7 ± 5.7% in Halibut Cove. In Seldovia, Halibut Cove and Port Graham transplants lost four individuals over the summer. There was no significant difference in temporal transplant survivorship between study sites (ANOVA for repeated measures; $df = 2$; $p > 0.05$). Transplant survivorship was not significantly affected by the origin of the plant (ANOVA for repeated measures; $df = 2$; $p > 0.1$).

2.4. – DISCUSSION

Kelps are marine algae that are not commonly found in estuaries because of their low tolerance to hyposalinity (Scagel 1961; Druehl 1981; Dawson and Foster 1982). In addition to a progressive decrease in salinity along the estuary axis, the amount of suspended particles from land, rivers, and glacial run-off increase toward the head of inlets. The geographic and depth distribution of algae has been observed to be restricted by increasing turbidity levels (Edwards 1980; Lumb 1989). The distribution of *Nereocystis* kelp beds in Kachemak Bay appears to follow this paradigm. The hydrographic data collected during this study support the idea that there is a salinity and turbidity gradient along the south axis of Kachemak Bay. Surface salinity is significantly lower at the inner bay site than at the mid-bay and outer bay sites but bottom salinity is less different among sites. Turbidity is higher throughout the water column and light attenuation is greater in Halibut Cove than in Port Graham and Seldovia. The water column is stratified in Halibut Cove but better mixed in Port Graham and Seldovia.

It was expected that conditions of low salinity and high turbidity found in estuarine environments would limit growth and survival of plants transplanted from the outer bay. The results of transplantation experiments from an oceanic to an estuarine environment indicated that there was a negative estuarine effect on specific growth rates of *Nereocystis* sporophytes. Specific growth rate of plants collected from Port Graham were progressively slower as they were transplanted to sites closer to the head of the bay. Reduced salinity has been reported to stunt algal growth in other studies (den Hartog 1971; Bolton 1979; Coudret *et al.* 1983; Gerard *et al.* 1987). The amount of light measured throughout the study in Halibut Cove was lower than in Port Graham and Seldovia and is also believed to contribute to the lower growth rate of Port Graham transplants in Halibut Cove than in Port Graham. Many authors have established a direct effect of irradiance on algal growth (Kain 1965; Kain *et al.* 1975; Drew and Jupp 1976; Lüning 1981a, b). Specific growth rates of Port Graham transplants appeared to be slower in Halibut Cove than in Seldovia. Unless light intensity reaches saturation and inhibition levels, the relation between light intensity and rate of photosynthesis is linear (for review see Ramus 1981). The negative estuarine effect observed on Port Graham plants was only marginal for Seldovia plants transplanted to Halibut Cove and is thought to result from more comparable hydrographic parameters at depth between the two sites.

In addition to a negative estuarine effect on outer bay transplants, a positive oceanic effect was expected for inner bay transplants, but this was not supported by this study. Higher salinity and light availability in Port Graham were anticipated to promote kelp growth (Duncan 1973; Bolton 1979; Gerard *et al.* 1987); however inner bay transplants did not grow significantly faster when transplanted to an oceanic site. Seldovia and Halibut Cove plants transplanted to the oceanic site did not grow faster under putatively better growing conditions. Based on results from this study, it is tempting to speculate that plants may be programmed to grow at a maximum rate that cannot be exceeded even under optimal growing conditions and that when transplanted to their site of origin or to habitats where conditions are better than at the

original site, *Nereocystis* plants reach a maximum SGR. In some cases, relative hypersalinity can have a negative impact on algal growth and photosynthetic rates (Greenway and Munns 1983; Herbst and Castenholz 1994); however, this did not occur in the present study, as estuarine plants were not impaired when transplanted to the outer bay.

The neutral response of plants transplanted from a site of marginal to a site of similar or better growing conditions suggests that plants from different origins are adapted to best grow under the conditions at their original site. Several studies suggest that salinity and light levels different from where the individual grew has a negative impact on algal growth (Munda 1961; den Hartog 1971; Gerard *et al.* 1987). Specific growth rates of plants from diverse origins exhibited disparate levels of correlation with increasing temperatures and may imply that plants from different sites respond variably to changes in some environmental factors. Port Graham plants responded more strongly to increasing temperature than Halibut Cove plants. Plants from Port Graham (and to a certain extent Seldovia) grew under relatively stable temperature conditions whereas plants from Halibut Cove were under variable temperature regimes. Adaptation or acclimation of a species to salinities (or other variables) higher or lower than normal may result from genetically diverse populations or through epigenetics (Yarish *et al.* 1979a, b; Innes 1984; Gerard and DuBois 1988; Innes 1988). It is difficult to disentangle the cause of phenotypic variability, especially in a wild population of unknown genotype because both polygenic and environmental factors can be responsible.

No genetic variability studies of kelp populations in Kachemak Bay have been conducted. However, results from this study indicate that *Nereocystis* sporophytes from different study beds have some flexibility in salinity, light, and turbidity tolerance depending on conditions of their original site. The cause of this adaptability is unclear, but SGR appeared to improve with time. When *Nereocystis* plants were transplanted to a site with marginal conditions in reference to their original site, the SGR started relatively slow but eventually increased. A maximum SGR may have been more perceptible if stipe growth had been monitored longer. The delayed response was particularly visible for Port Graham plants transplanted to Seldovia and Halibut Cove. The SGR of Port Graham plants in Port Graham increased steadily from the start of the experiment whereas it took four weeks in Seldovia and five weeks in Halibut Cove to initiate detectable SGR increases. By week 6, the SGR of Port Graham plants transplanted to Halibut Cove was not significantly different from that of resident Halibut Cove plants. In another study, *Laminaria saccharina* growth rate was slowed by salinity levels lower and higher than that of its original site but this inhibition was temporary; after enough time under the new conditions the plants acclimated and their growth rates increased (Gerard *et al.* 1987). Several marine algal species can adapt to salinity conditions dramatically different from that of their original site as long as the change is not abrupt (Hurd 1916; Geesink 1973). The adaptation of Port Graham plants transplanted in Halibut Cove may have been favored by conditions at depth (in contrast to the surface) being comparable to conditions in Port Graham and Seldovia.

Consequently, the shock for transplants from oceanic conditions was minimized and outer bay transplants had time to adapt before penetrating the upper water column fresh water layer.

Most transplants, from all origins and at all sites, displayed a period of fast increasing SGR, preceding a period of leveled SGR. Because control transplants also showed an increase, it is believed that in addition to the delayed SGR increase caused by an adaptation period and the maximum due to a potential programmed growth rate, a combination of other factors may dictate *Nereocystis* growth pattern. *Nereocystis* stipe elongation is influenced by factors such as depth, light quality and quantity (Foreman 1970), salinity, temperature, and nutrient concentrations (Duncan 1973), with spectral irradiation thought to be the greatest influence (Duncan 1973). Plant growth is often positively correlated with increasing light intensity, until saturation light levels are reached. In this study, a negative correlation was observed between SGR and light levels. This pattern may be misleading since the amount of light decreased as summer progressed due to shorter days and this relationship may be merely a correlation, not a causal relationship. Moreover, as time passed, the plants grew closer to the surface and received more light than was measured at 8 m MLLW. Specific growth rates increased as plants grew taller. *Nereocystis* sporophytes have three primary regions of growth, upper stipe, holdfast, and base of the blades (Nicholson 1970), but most of the thallus has some diffuse growth capacity (Nicholson 1970; Kain 1987). As a result of diffuse growth, more growth can occur and a greater specific growth rate is recorded as the stipe elongates. Specific growth rates of *Nereocystis* stipes were relatively low (between 0.3 and 2.3 cm/day on week 2) at the beginning of the experiment but eventually increased to reach 9 cm/day on week 5 and 6 (for Seldovia transplants in Seldovia and Halibut Cove). After a rapid elongation period, many plants ultimately reached a maximum SGR after week 5. Similar patterns of rapid SGR followed by curtailed rates have been observed by other authors (Foreman 1970; Duncan 1973; Maxell and Miller 1996). It was postulated that the pattern of stipe elongation was controlled by a phytochrome response to changes in the ratio of red to far-red wavelengths (R:FR) throughout the water column (Duncan 1973). Duncan and Foreman (1980) suggest that far-red radiation rather than the increase of total PAR is responsible for onsetting *Nereocystis* rapid stipe growth rates. Lüning (1980) found similar effects on *Laminaria saccharina* stipe elongation. Both red and far-red radiation are selectively absorbed by water, with far-red being attenuated more rapidly and causing R:FR to increase with depth. The rapid change in R:FR usually occurs below 2 meters from the surface, but because the rates of absorption of different wavelengths depend on water clarity and the type of suspended particles present, the extent and depth at which the rapid changes in the ratio occurs is frequently altered by tidal activities and turbidity levels (Duncan and Foreman 1980). A rapid SGR was observed in most transplants, but occurred at approximately 5 m depth, well before the plants reached a depth of 0 m MLLW. This phenomenon may be explained by the extreme tidal range in Kachemak Bay allowing plants to be frequently exposed at the surface before they reach 0 m MLLW.

The great tidal range in Kachemak Bay also may contribute to the growth pattern observed in natural *Nereocystis* populations surrounding the transplants (personal observation). It has been shown that *Nereocystis* considerably slows its stipe growth once sporophytes reach the surface (Foreman 1970; Duncan 1973). This was not observed in the present study since the experiment was terminated before transplants reached the surface (4 m from 0 m MLLW), but it appeared to occur in the natural *Nereocystis* populations surrounding the transplants. Toward the end of summer, most mature plants had not reached the surface, even at low tide. It is postulated that because of the great tidal range in Kachemak Bay, plants may receive enough light to grow and start allocating energy to reproduction without having reached the surface. McAlary and McFarland (1994) noticed that in California, *Macrocystis* sp. blades deteriorate at the surface due to exposure to higher temperatures. In Kachemak Bay, a surface layer of fresher, warmer water develops in summer, particularly in the inner bay, and it is possible that this barrier hinders further growth.

Transplant survivorship remained relatively high during the six weeks of the experiment. Most casualties occurred at the beginning of the experiment and are thought to be related to handling stress. Some mortalities, especially in Seldovia, are believed to be caused by a grazing snail, *Lacuna vincta* (see Chapter 4). Since there was no significant difference in survivorship based on origin or site of study, it is not thought that salinity and turbidity regimes had a significant impact on *Nereocystis* survival. Survivorship was highest in Halibut Cove where environmental conditions were considered marginal. The levels of salinity, turbidity, and light attenuation in this study were not extreme enough to cause mortality. The lowest PAR intensity measured in Halibut Cove was 11.8 $\mu mol/m^2/s$, which is above the minimum amount of light required for *Macrocystis pyrifera* growth (ca. 4.6-8.1$\mu mol/m^2/s$ according to Dean and Jacobsen 1984). Also, if the high-turbidity, and low-light conditions are cyclic, *Nereocystis* sporophytes may adjust to momentary peaks of low light as they can grow in the dark for short time periods by using photosynthate translocation (Schmitz and Lobban 1976; Nicholson and Briggs 1972; Kain 1987) and light-independent carbon fixation process (Schmitz 1981). *Nereocystis* contains elongated sieve cells that are specialized in solute transport from regions of active photosynthesis (such as blades and the upper stipe) to apical and basal intercalary meristems (Nicholson and Briggs 1972; Schmitz and Lobban 1976). This process allows plants to take advantage of low light attenuation toward the surface and to keep growing even though the lower portion of their thallus is only receiving a limited amount of light. Many brown algae, including *Nereocystis*, are capable of maintaining positive net production in the dark via light-independent carbon assimilation (Schmitz 1981). Dark carbon fixation can account for over 20% of total carbon fixation in brown algae (versus less than 3% in green and red algae). Brown algae are not classified as C_3 or C_4 plants because of their unique combination of photosynthetic and dark carbon assimilation (Schmitz 1981). In Kachemak Bay, the water column height and water clarity oscillate with ebbing and flooding tides, allowing plants in Halibut Cove to be periodically exposed to higher light levels. Intermittent high levels of turbidity may not be as detrimental as lower levels of continuous turbidity.

Nereocystis, similar to other kelps, is sensitive to high temperatures and in British Columbia and northern Washington can be irreversibly damaged if water temperatures reach 18° C (O'Clair and Lindstrom 2000). The highest temperature recorded during this *in-situ* experiment was 12.8° C and well below *Nereocystis* tolerance limit. Survivorship, as an ecological parameter, has been found to be fairly insensitive to most environmental fluctuations compared to growth and biomass measurements (Tegner *et al.* 1996).

2.5.CONCLUSION

Nereocystis individuals transplanted from an oceanic to an estuarine environment showed decreased growth rates. No lethal effect of the estuarine conditions on the survival and growth of *Nereocystis* sporophytes was observed. The limited negative impact of estuarine conditions on plants collected from the outer bay and transplanted in the inner bay may be caused by inner and outer bay conditions being comparable at 8 m MLLW. A more detrimental impact would be expected if the outer bay plants were transplanted closer to the head of the bay where waters are fresher and more turbid. It is difficult with field studies to disentangle the role of each individual factor as salinity, light attenuation, turbidity, and temperature vary simultaneously and may co-vary. Laboratory experiments on *Nereocystis* sporophyte growth under controlled treatments would help determine the importance of the different factors. In addition, other factors, such as nutrient concentrations, wave energy and current regimes, and sedimentation may have critical effects on the growth and distribution of *Nereocystis* in Kachemak Bay. Although plants may be adapted to the local environmental conditions under which they grew, *Nereocystis* plants in Kachemak Bay may be able to acclimate to more marginal conditions as long as the changes are subtle and progressive. Ecologically, unless the conditions change dramatically and abruptly (such as point source pollution), kelp beds are believed to be somewhat robust to changes in salinity, turbidity and light conditions. Because estuarine conditions as measured in Halibut Cove are not detrimental to adult *Nereocystis*, the distribution of the kelp beds in Kachemak Bay is not thought to be dictated only by the fitness of the sporophytes. It is proposed that the critical factor may be the effects of low salinity and light intensity on microscopic life stages (see Chapter 3) or may be related to spore dispersal ability (Carl Schoch, KBRR, in preparation).

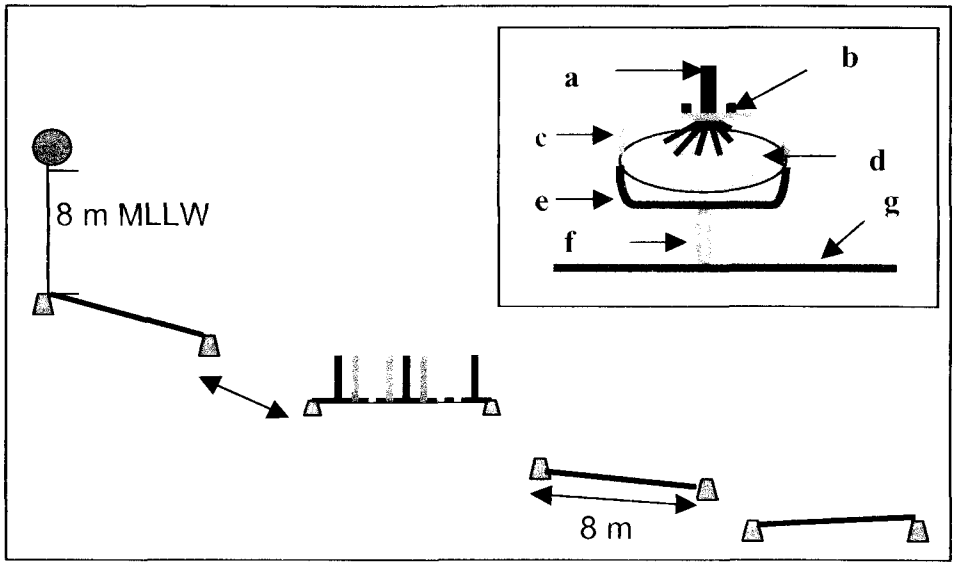

Figure 2.1: Design used to transplant kelp at each site. Four 8-m long ground lines were placed 5 to 10 m apart at a depth of 8 m MLLW. Three transplants from each origin (Port Graham, Seldovia, and Halibut Cove) were randomly placed 50 cm apart on each line. The schema in the insert represents the method used to attach each transplant on the ground lines (a) Kelp stipe and holdfast, b) cable ties, c) soft nylon line, d) small polystyrene float, e) polypropylene line, f) gagnion hook, g) ground line).

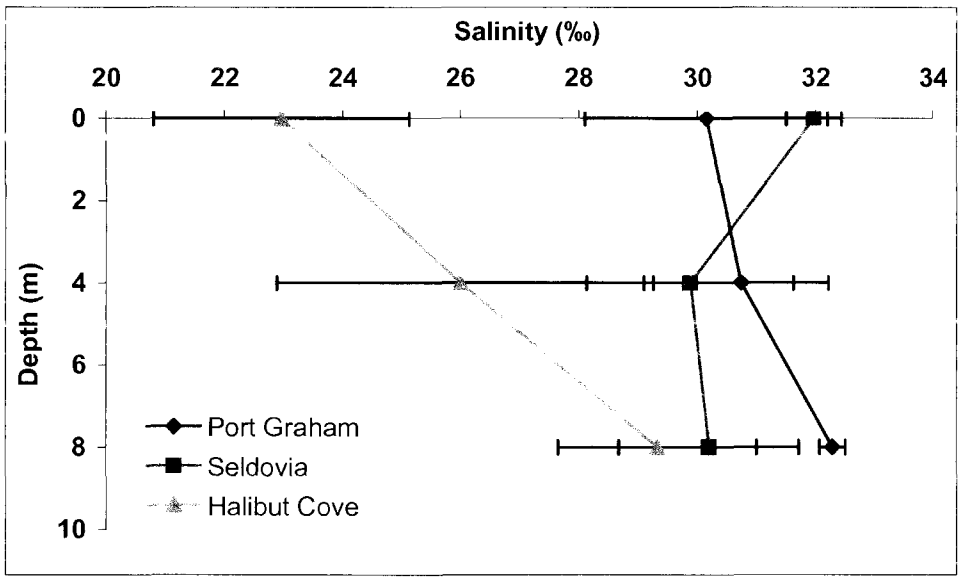

Figure 2.2: Salinity profile at each study site based on water samples collected at three depths on three occasions during Summer 2001. Samples were analyzed in the laboratory with a YSI Instrument. The values represent the means (± 1 SE) (n = 3).

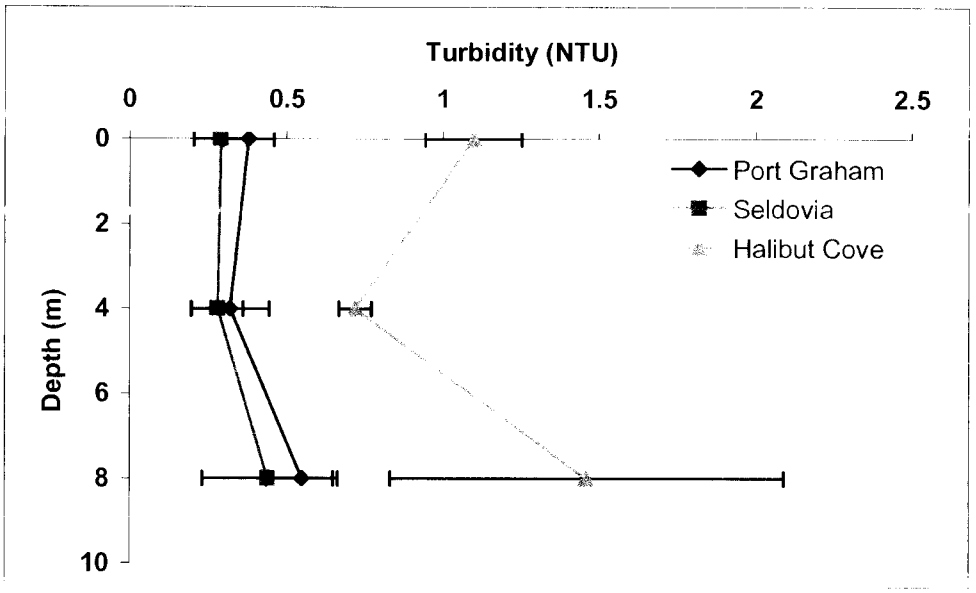

Figure 2.3: Turbidity profile at each study site based on water samples collected at three depths on three occasions during Summer 2001. Samples were analyzed in the laboratory with a LaMotte turbidimeter (units in NTU). The values represent the means (± 1 SE) (n = 3).

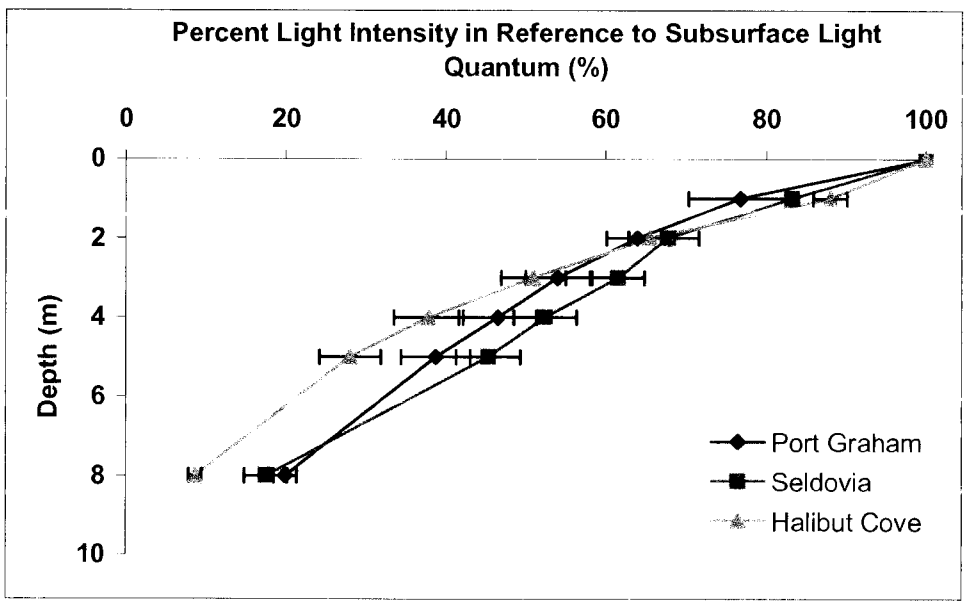

Figure 2.4: Light intensity profile at each study site based on three measurements during Summer 2001. The measurements were made with a Li-Cor PAR light meter (units in $\mu mol/m^2/s$). The values represent the percentage of PAR measured at each depth in reference to the quantum radiation measured right below the surface. The values are means (± 1 SE) (n = 3).

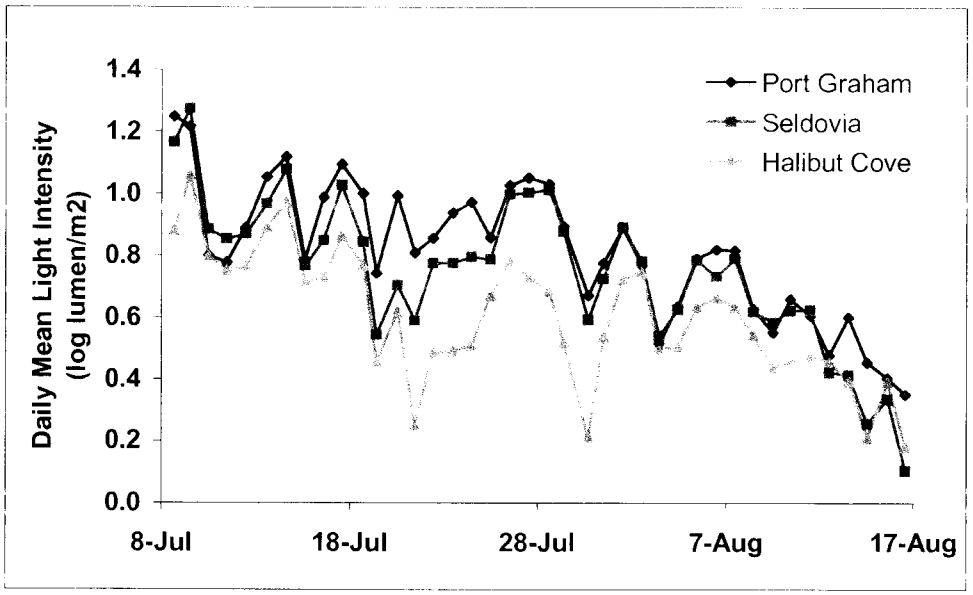

Figure 2.5: Variation in mean daily light levels (log lumen/m^2) at a depth of 8 m MLLW throughout the duration of the study. The means are based on hourly readings averaged over a 24-hour period.

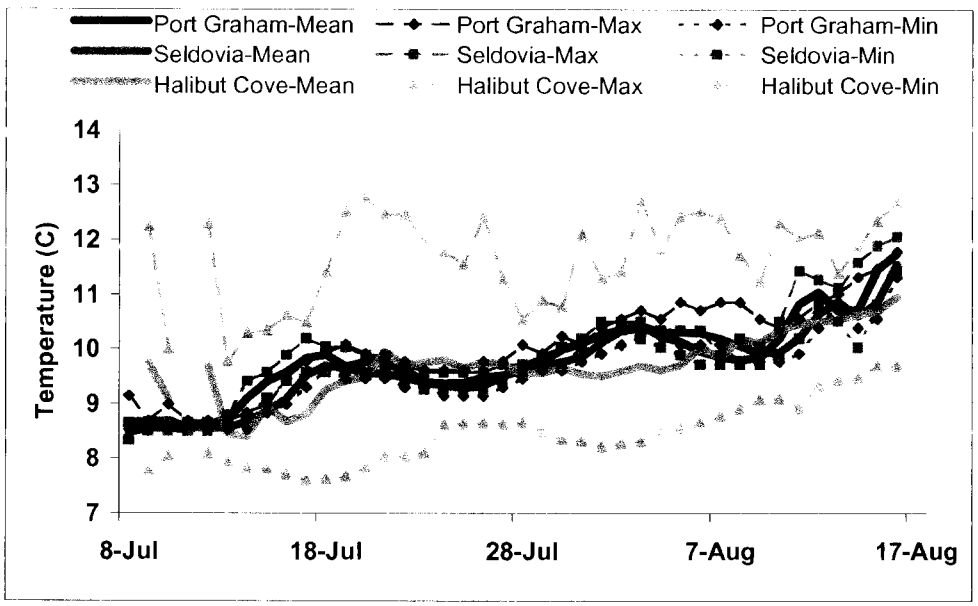

Figure 2.6: Variation in daily temperatures (° C) at a depth of 8 m MLLW throughout the duration of the study. The means, minima and maxima are based on measurements made every ten or fifteen minutes. The means are represented by bold lines, the minima by dotted lines, and the maxima by dashed lines.

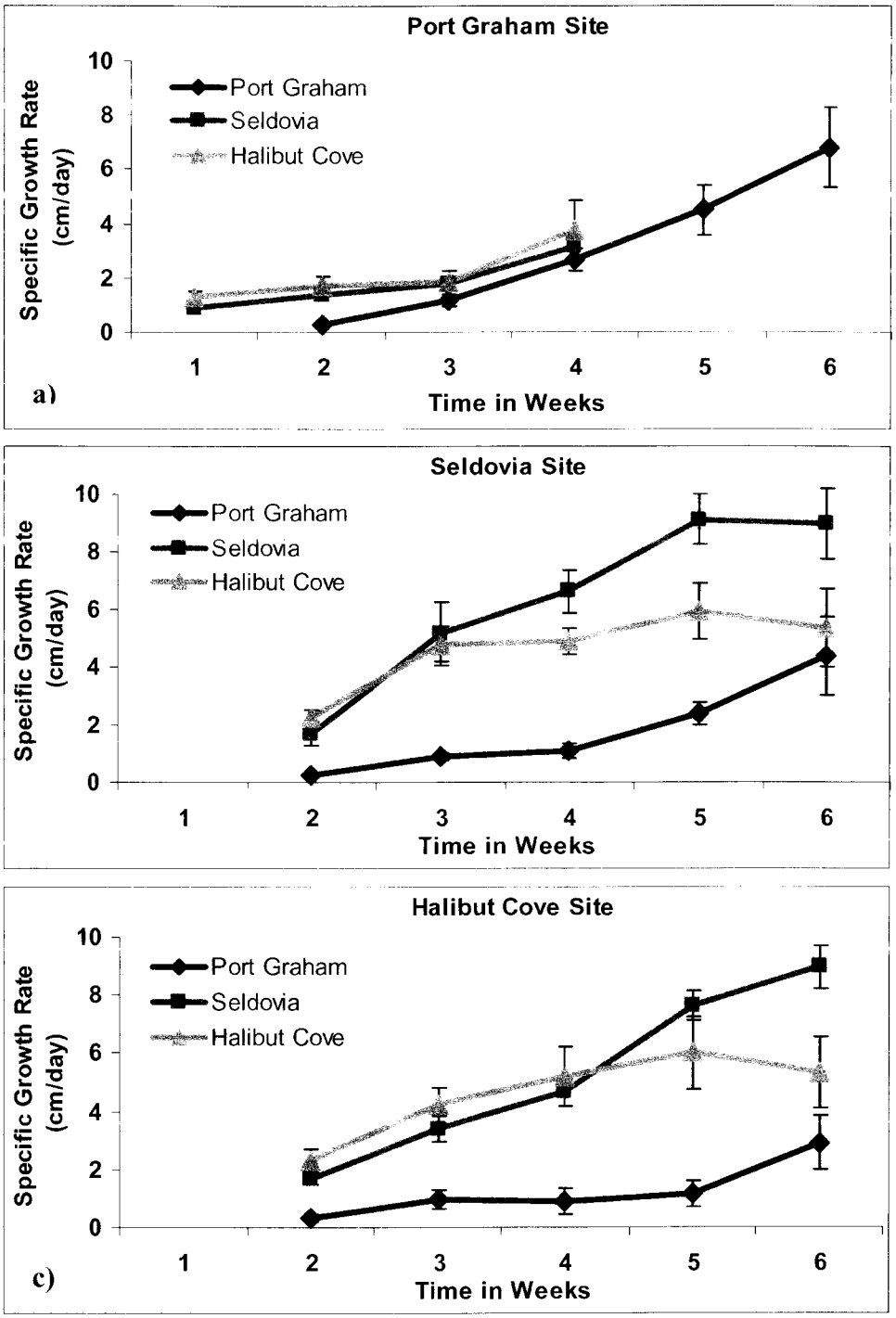

Figure 2.7: Specific growth rates (cm/day) of *Nereocystis* plants collected from Port Graham, Seldovia, and Halibut Cove and transplanted to: a) Port Graham, b) Seldovia, and c) Halibut Cove. The values represent the mean specific growth rate (± 1 SE) measured over four to six weeks during Summer 2001. The number of individuals for each category varied from twelve to eight due to the loss of some transplants over the summer.

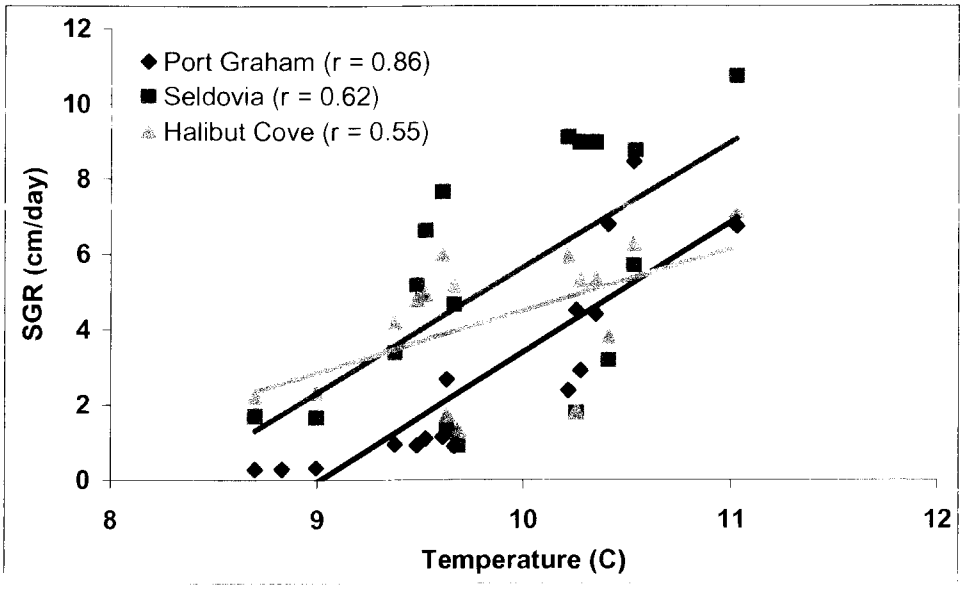

Figure 2.8: Specific growth rate (cm/day) versus mean temperature (° C). The daily temperature means were averaged over the period separating the sampling events at each study site. The correlation is calculated separately for transplants from different origins.

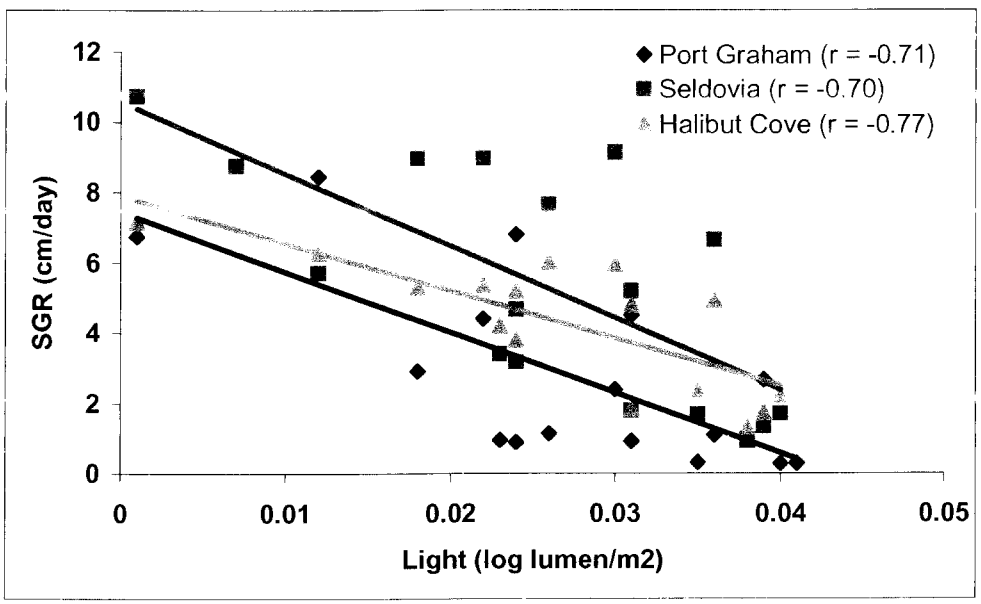

Figure 2.9: Specific growth rate (cm/day) versus mean light levels (log lumen/m^2). The daily light means were averaged over the period separating the sampling events at each study site. The correlation is calculated separately for transplants from different origins.

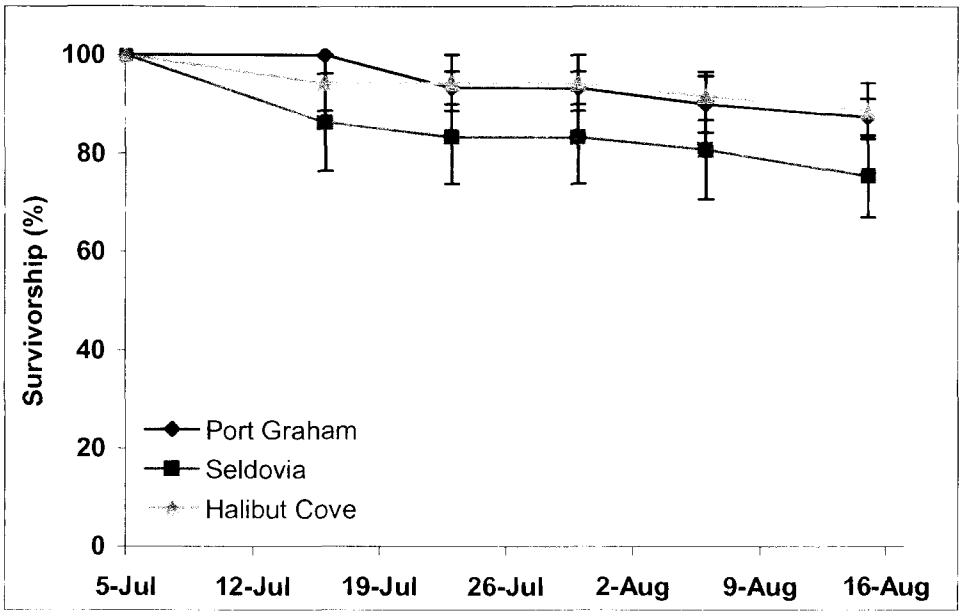

Figure 2.10: Survivorship of transplanted *Nereocystis* at each study site throughout Summer 2001. The survival rate is calculated as the percentage of individuals alive on each census date in reference to the original number of plants transplanted at each site. The means and SE are based on survivorship across all origins at each site.

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CHAPTER 3
EFFECT OF SALINITY AND LIGHT INTENSITY ON THE DEVELOPMENT OF *NEREOCYSTIS LUETKEANA* SPORES

3.1 - INTRODUCTION

The life history of *Nereocystis luetkeana* contains conspicuous sporophytes alternating with a microscopic gametophytic generation. Despite their elusive nature, kelp propagules are extremely important to the subsequent success and distribution of adults. Numerous studies have suggested the importance of the successful development of cryptic life stages for the success of individual adults (Kain 1964, 1965; Vadas 1972; Dean and Deysher 1983) and entire kelp populations (Fletcher and Callow 1992; Vadas *et al.* 1992; Doblin and Clayton 1995; Kevekordes 2000; Kevekordes and Clayton 2000). Propagule survival and successful dispersal also are crucial to enhancing genetic diversity and distribution range of algal species (Dean and Jacobsen 1984; Reed 1987; Norton 1991; Clayton 1992; Norton 1992a; Reed *et al.* 1992; Graham 2000).

The cryptic nature of microscopic stages makes them more challenging to study than conspicuous sporophytes. Because of the dramatic size difference between kelp macro- and microscopic stages, they inhabit different physical environments (primarily within the water column and the boundary layer, respectively) (Neushul 1981a; Amsler *et al.* 1992; Norton 1992a). Consequently, the various ontogenetic stages might be affected by different factors and have different physiological requirements (Foster and Schiel 1985; Chung and Brinkhuis 1986). The microscopic stages suffer high mortality rates and are thought to be the bottleneck phase (Santelices 1990; Fletcher and Callow 1992; Norton 1992b; Vadas *et al.* 1992; Graham 2000). Because of their enhanced sensitivity to various environmental stresses and toxicants, microscopic developmental stages are often used for toxicity bioassays (Anderson and Hunt 1988; Anderson *et al.* 1990; Wright and Reed 1990; Reed and Lewis 1994; Reed *et al.* 1994; Doblin and Clayton 1995; Burridge *et al.* 1999a, b; Kevekordes and Clayton 1999, 2000; Kevekordes 2000).

Once released in the water column, spores must complete a series of distinct developmental steps to successfully transform into gametophytes (Amsler *et al.* 1992; Clayton 1992; Fletcher and Callow 1992). Swimming spores can make active behavioral choices to settle within a microenvironment (Amsler *et al.* 1992, 1999). Once an appropriate settlement location is encountered, the spore initiates attachment at the tip of its anterior flagellum (Goldstein 1992). The two flagella are then withdrawn into the cell body and an adhesive is secreted (Henry and Cole 1982). Once the spore is attached, the cell wall is secreted. Spore adhesion takes up to fifteen hours to reach maximum strength on glass (Charters *et al.* 1971, 1973; Neushul 1981b; Amsler *et al.* 1992). Initiation of germination and germ tube protrusion from the spore cell is associated with translocation of cytoplasm and organelles (Pillai *et al.* 1992). Based on the development of

spores, various parameters such as sinking tendency, settlement success, germination success, and germ tube length can be investigated to determine spore vulnerability to environmental factors.

Some environmental factors known to affect kelp propagules include salinity and light intensity and quality. Several studies have shown the detrimental effects of reduced salinity on spore germination, gametophyte development and fertilization, and embryo growth in various macrophyte taxa (Norton and South 1969; Druehl and Hsiao 1977; Anderson and Hunt 1988; Wright and Reed 1990; Anderson *et al.* 1992; Doblin and Clayton 1995; Burridge *et al.* 1999a, b; Stekoll *et al.* 1999; Kevekordes 2000; Kevekordes and Clayton 2000). Elevated salinity also can have deleterious effects on some algal spores causing developmental abnormalities, substantial decrease in growth, and physiological alterations (Young *et al.* 1987a, b; Wright and Reed 1990; Herbst and Castenholz 1994). Spores are more susceptible than adult plants to changes in salinity because they lack a cell wall (Henry and Cole 1982; Russell 1987). If osmotic adjustments are not made by the spore in response to increasing or decreasing salinity, the cell membrane, organelles, and enzymes can be damaged (Gessner and Schramm 1971; Reed *et al.* 1980; Russell 1987).

Light can trigger specific developmental stages and influence kelp reproduction and sporophyte growth (Vadas 1972; Lüning and Dring 1975; Lüning and Neushul 1978; Lüning 1980, 1981a, b, c; Dean and Jacobsen 1984; Deysher and Dean 1984, 1986a, b). Many investigations have examined the role of light on post-settlement stages (gametophytes, zygotes, and microscopic sporophytes); fewer have investigated the effect of light on spores. The settlement success of the brown alga *Hincksia irregularis* improved in the dark (Greer and Amsler 2002) while the mobility of *Macrocystis pyrifera* and *Pterygophora californica* spores was prolonged under saturating irradiance (Reed *et al.* 1992). Spores of several algal species, including *Nereocystis*, have photosynthetic capabilities that contribute to their own nutrition (Amsler 1988a; Amsler and Neushul 1991). Excessive irradiance can, in turn, be detrimental to *M. pyrifera* spores by limiting spore settlement and inhibiting gametophyte and embryonic sporophyte survival (Graham 1996). Ultra-violet radiation, in particular, plays an important role in inhibiting *M. pyrifera* recruitment by disrupting nuclear division and translocation events that follow spore germination (Huovinen *et al.* 2000).

In Kachemak Bay, the distribution and density of *Nereocystis* beds decrease along the axis of the bay and appear to follow salinity and turbidity gradients, with no *Nereocystis* beds found at the head of the bay where conditions are brackish (Figure 1.2). Drifts of fertile *Nereocystis* sporophytes occasionally penetrate in the inner bay (Carl Schoch, KBRR, personal communication). Drifts may be a means for kelp to invade and establish themselves in new areas (Dayton *et al.* 1984; Fletcher and Callow 1992; Norton 1992a; Reed *et al.* 1992, 2000; Bushing 1994). However, in Kachemak Bay, drift plants do not seem to be successful at establishing new beds in the inner bay. If spores from drift plants are viable, it is thought that salinity and light attenuation, associated with turbidity, may be influencing the overall distribution of

Nereocystis kelp beds in Kachemak Bay by preventing spores from establishing themselves in the inner bay's brackish waters. Depending on their location in the bay, kelp beds are subject to different salinity and turbidity/light conditions and kelp individuals from different sites may be adapted to the local conditions of their original site. In addition, *Nereocystis* is usually considered an annual although some individuals can persist for a second growing season (Foreman 1970; Nicholson 1970; Chenelot *et al.* 2001; Duggins *et al.* 2001). In Kachemak Bay, adult plants can survive the winter, grow new blades and become reproductive the following summer (personal observation). Spores of second-year plants examined in central California appeared 'normal in every respect' but no further details on the criteria used to evaluate spore fitness were given (Foreman 1970).

The main objective of this study was to assess the potential effects of salinity and light intensity on *Nereocystis* spore development by monitoring the sinking tendency, settlement success, germination success, and germ tube length of spores under different salinity and light levels after 24 hours of incubation in the laboratory. The comparison between spores collected from an oceanic (Port Graham) versus an estuarine site (Halibut Cove) also was examined to elucidate any possible local acclimation or adaptation. In addition, the fitness of spores produced by second-year versus first-year plants was compared.

3.2 – METHODS

3.2.1. - Collection sites

Spores were collected from ripe sporophytes at three different sites within Kachemak Bay, Alaska (Figure 1.2). The oceanic site, Port Graham, is located in the outer bay and is characterized by high salinity and low turbidity water. The estuarine site, Halibut Cove, is located in the inner bay and is characterized by brackish water. Seldovia is located between the two distant sites and has intermediate hydrographic characteristics.

Laboratory experiments to examine the effects of salinity and light intensity on the development of *Nereocystis* spores were conducted separately using four different groups of spores: 'PG' spores were collected from Port Graham and 'HC' spores from Halibut Cove; 'Old' and 'New' spores were collected in Seldovia from second-year and first-year plants, respectively. The term 'distant spores' refers to PG and HC spores, collected respectively from the oceanic and estuarine site; 'Seldovia spores' is the general term for Old and New spores.

3.2.2. - Spores

For each experiment, approximately 25-30 blades carrying ripe sori were collected from at least ten mature *Nereocystis* sporophytes. Cut blades were transported to the laboratory in a cooler filled with seawater and were kept in running seawater (27-28‰) at the laboratory until they were used (within 48

hours after collection). A new batch of sori was used for each experiment. The protocol for spore release was based on Stekoll and Else (1990). Sori were brushed gently and rinsed with 5-µm filtered seawater to reduce contamination by microscopic epiphytes. The sori were blotted dry, laid between paper towels and newspaper, covered with plastic wrap and placed in a dark refrigerator at 5° C for thirty minutes to four hours (depending on sori ripeness). The thermal and light shock promoted a more successful and synchronized spore release. Approximately 10-15 sori were rinsed to wash away the prematurely released spores, immersed in 1 L of 0.45-µm filtered seawater and left undisturbed in an incubator (ca. 9° C; 55 µmol/m^2/s) until the solution darkened. If no spores were released after sixty minutes, a new batch of sori was used. Once enough spores had released (which took from five to sixty minutes depending on sori ripeness), the spent sori were discarded and the container was left undisturbed in the incubator for another thirty minutes to let the mucilage and other impurities settle. The spore solution was filtered through four layers of cheesecloth and the concentration of released spores was estimated using a hemocytometer. To slow down the spores and allow more accurate counts, 1 mL of glacial acetic acid was added to 9 mL of spore solution pipetted from the middle of the water column. Spore concentrations were multiplied by 1.111 to correct for dilution with the acetic acid. Spore concentrations varied for each spore release, ranging from 225,000 to 550,000 spores/mL. The concentrated spore suspension was used to inoculate the different treatments so that the final concentration was 5,000 spores/mL. The following formula was used to calculate the proper volumes needed to obtain the desired final spore concentration:

$$\mathbf{V_S = (5{,}000 * V_f) / C_S}$$

V_S: volume of concentrated spore suspension

C_S: spore concentration in concentrated spore suspension

V_f: volume of final culture

During each experiment, spores were subjected to either salinity or light treatments (Figure 3.1). Three salinity levels (20‰, 27‰, and 35‰) or four light intensities (0, 18, 55, and 135 ± 5 µmol/m^2/s) were used. The spores used for the salinity treatments were grown under a light intensity of 55 µmol/m^2/s, while the spores used for the light treatments were grown in 27‰ seawater. The set of petri dishes combining a salinity of 27‰ and light level of 55 µmol/m^2/s (Control) was shared by both salinity and light treatments. Each treatment was filled with 15 mL of adjusted spore solution and was replicated five times. The spores that settled on circular microscope cover slips, placed in pairs in the plastic petri dishes. Only one cover slip was used for each count, the second was available if necessary to allow the number of replicates to stay constant (n=5).

3.2.3. - Saline solutions:

Brine was produced by freezing and partially thawing filtered seawater. Solutions of desired salinity were produced by mixing either brine (60-75‰) or thawed ice from the brine-making process (15-22‰) with filtered seawater (at 27-28‰) or with dionized water. Salinities were verified using a hand-held refractometer. The following formulas were used to calculate the various volumes needed:

$V_E = V_B\ [(S_B - X) / (X - S_E)]$

V_B: volume of brine
V_E: volume of filtered seawater
S_B: salinity of brine
S_E: salinity of filtered seawater
X: desired salinity

$V_F = V_E + V_B$

V_F : final volume of desired solution

3.2.4. – Incubation

Desired irradiance levels were obtained in the incubator by adjusting the distances between the light source (four 50-Watt white fluorescent tubes) and the petri dishes and by shading petri dishes with various grades of window screen (based on Deysher and Dean 1986b). A light meter (Li-Cor, model LI-250) with a spherical PAR qantum sensor (Li-Cor, model LI-193SA) was used to determine light intensity throughout the incubator and to find the correct combination of screen layers and distance from the light source to obtain the desired light levels. Petri dishes assigned to 0 $\mu mol/m^2/s$ were wrapped in black plastic. Petri dish locations of each light level could only be randomized within themselves because few spots were available for each specific light intensity. A temperature logger (Onset, Model HOBO) placed in the incubator monitored temperature fluctuation every ten minutes.

3.2.5. – Scoring methods

Scoring petri dishes began 22 hours after incubation. Lights in the incubator were turned off when the first petri dish was removed. The order in which the petri dishes were scored was random for each experiment and the time each replicate was removed from the incubator was recorded so that the exact incubation time could be calculated.

To standardize the amount of water remaining (or the thickness of the boundary layer in which counted spores were trapped) on all cover slips, excess water was absorbed by touching the cover slip rim on a paper towel. The slip was examined under a phase-contrast microscope with 40 x 10 magnification and all spores seen within five fields of view were counted and scored. The fields of view were haphazardly selected but were kept away from the cover slip rim to avoid potential edge effects. Non-germinated and

germinated spores were counted separately, with germination being defined by the presence of a germ tube at least as long as the diameter of the spore (ca. 4-5 µm). Loose spores were then washed away by squirting filtered seawater on the cover slip (Reed *et al.* 1992). Once loose spores were removed, the remaining settled spores (both non-germinated and germinated) were counted.

Spore sinking tendency represents the relative fraction of spores that reached the boundary layer 24 hours after being added to the Petri dishes. Sinking tendency was assessed by counting spore numbers before rinsing the cover slip and was scored separately for non-germinated (SinkNG) and germinated (SinkGerm) spores. Sinking tendency was expressed in number of spores. Settlement success was estimated for non-germinated (SSNG) and germinated (SSGerm) spores by comparing spore numbers before and after rinsing and was expressed in percentages of spores originally counted within the boundary layer (before rinsing; SinkNG and SinkGerm, respectively). Germination success was determined for settled spores only (GSSett) and was expressed in percentages of adhered spores that had grown a germ tube. To measure germ tube length (GTL), ten germinated, settled spores with straight germ tubes were haphazardly selected and their germ tube measured. Germ tube length was measured as the distance between the outside of the circular spore cell to the tip of the germ tube. Figure 3.2 displays the different parameters studied and the scoring procedure steps.

3.2.6. – Statistical analysis

Sinking tendency and settlement success numbers appeared different for non-germinated versus germinated spores, therefore, t-tests were performed to validate estimating sinking tendency and settlement success separately for non-germinated and germinated spores. T-tests on paired observations compared sinking tendency of non-germinated versus germinated spores and settlement success of non-germinated versus germinated spores for each spore type.

Nested, mixed-model ANOVAs (SAS Institute Inc., 1999) were used to determine the effects of salinity and light on the development of *Nereocystis* spores from different origins (Port Graham, Halibut Cove) and of different ages (Seldovia-Old, Seldovia-New). Four ANOVA's were run separately for distant spores (PG and HC) under salinity and light treatments, and for Seldovia spores (Old and New) under salinity and light treatments. The response variables were spore sinking tendency (SinkNG and SinkGerm), settlement success (SSNG and SSGerm), germination success (GSSett), and germ tube length (GTL). Experiments were nested within (type*treatment), with type referring to spore origin (Port Graham or Halibut Cove) or age (Old or New) and treatment referring to salinity or light as experimental variables. The effects 'type', 'treatment', and 'type*treatment' were fixed but the 'experiment (type*treatment)' effect was random and used as the error term. Because of the use of a mixed model, the Satterthwaite Approximation was applied to estimate the F statistics (SAS Institute Inc., 1999). The data are presented using least-square means (LS means) and standard errors based on the Satterthwaite method. Three

replicate experiments, each using a unique batch of spores and done on different dates, were conducted for each spore type (PG, HC, Old, or New). Each treatment (20‰, Control, 35‰, 0 µmol/m^2/s, 18µmol/m^2/s, 135 µmol/m^2/s) was replicated five times within each experiment and the counts from all five petri dishes were combined to one mean value per experiment and treatment. In an effort to limit variance amongst replicate experiments due to temperature fluctuation and amongst petri dishes due to different incubation time, temperature and time were included in the statistical model as covariates. Pearson correlations (r) between mean temperatures or incubation times and the values of the six studied parameters also were investigated (SAS Institute Inc., 1999).

To meet the homoscedasticity assumption, the data for sinking tendency, settlement success, and germination success were arcsine, square-root transformed. No transformation was necessary for germ tube length. The difference amongst the means within fixed factors was assessed and the statistical significance of difference was set at $\alpha = 0.05$; however, comparisons at a higher level of significance ($\alpha = 0.1$) will be noted as evidence of a weak statistical relationship. Comparisons between Port Graham and Halibut Cove, between Seldovia-Old and Seldovia-New, and amongst treatment levels were done by performing multiple comparisons among pairs of means using Tukey tests (SAS Institute Inc., 1999).

3.3 – RESULTS

3.3.1. – Temperature and time effects

Although incubator temperature was intended to be constant (10° C), it fluctuated throughout and between each incubation period (Table 3.1). Maximum and minimum temperatures ranged between 14.8° C and 8.2° C. The highest and lowest mean temperatures ranged between 12.9° C and 9.0° C. The greatest range of variation was 4.2° C during experiment HC 3. There was no systematic pattern in temperature variation for experiments with particular spore types. Correlations between mean temperatures and the study parameters showed some significant relationships ($p < 0.001$; Table 3.2) with SinkNG and GTL being positively correlated (r = 0.46 and 0.30, respectively) and SSNG and SSGerm being negatively correlated (r = -0.57 and -0.29, respectively) to mean temperatures. In contrast, SinkGerm and GSSett correlations to mean temperatures were non-significant (r = 0.04 and 0.03, respectively; $p > 0.1$). ANOVA results also indicated that mean temperatures had a significant effect on several parameters, especially germ tube length (Tables 3.3 and 3.4).

On average, spores spent 25 ± 1.8 hours in the incubator but because of the lengthy scoring procedure, individual incubation times varied between 21 and 29 hours. Only GTL showed a moderate correlation to incubation times (r = 0.28; Table 3.2).

3.3.2. – Non-germinated versus germinated spores

T-tests on paired observations suggested that non-germinated and germinated spores were dissimilar regarding sinking tendency and settlement success (Table 3.5). The sinking tendency and settlement success of germinated spores was significantly greater than that of non-germinated spores for distant (PG and HC) and Seldovia New spores, under both salinity and light treatments ($p < 0.05$; except for PG spores under control values and 35‰ treatments). The mean difference between SinkNG and SinkGerm for PG spores decreased from -0.38 at 20‰ to -0.16 at 35‰ because PG sinking tendency decreased with salinity. In contrast, sinking tendency and settlement success was significantly greater for non-germinated than germinated Old spores ($p < 0.05$). Seldovia Old spores had the highest and lowest sinking tendencies of all non-germinated (3.60) and germinated spores (1.14), respectively.

3.3.3. – Salinity effects

Salinity had a significant effect on some spore development parameters (Figures 3.3 and 3.4; Table 3.3 a and b). Salinity had no significant effect on sinking tendency and settlement success of both distant and Seldovia non-germinated spores. Sinking tendency indices (expressed in arcsine square-root transformed spore numbers) over all salinities were 3.1 ± 0.1, 3.1 ± 0.1, 3.1 ± 0.1, and 2.5 ± 0.1 for PG, HC, Old, and New non-germinated spores, respectively. On average, 26.9 ± 3.0%, 27.9 ± 3.0%, 21.9 ± 2.3%, and 45.4 ± 2.3% of PG, HC, Old, and New non-germinated spores, respectively, successfully settled at all salinities. Sinking tendency of germinated distant spores was not significantly affected by salinity (ANOVA; $F = 2.26$; $p = 0.1502$; Figure 3.3 a; Table 3.3 a). Sinking tendency of germinated Seldovia spores only showed a weak significant response to salinity (ANOVA; $F = 8.70$; $p = 0.0760$; Figure 3.4 a; Table 3.3 b) that was not supported by Tukey tests. Sinking tendency indices over all salinities were 3.3 ± 0.0, 3.6 ± 0.0, 1.1 ± 0.1, and 3.4 ± 0.1 for PG, HC, Old, and New germinated spores, respectively.

The settlement success of Seldovia germinated spores was significantly affected by salinity (Figure 3.4 b; Table 3.3 b) with SSGerm being significantly lower at 20‰ than at 35‰ (Tukey; $p = 0.0271$ and 0.0055 for comparisons between 20‰ and 35‰ and between 27‰ and 35‰, respectively). The germination success was significantly lower at 20‰ than at 35‰ for distant spores (Figure 3.3 c; Table 3.3 a). The germ tube lengths of all spores (distant and Seldovia) were significantly affected by salinity (Figures 3.3 d and 3.4 d; Table 3.3 a and b). Tukey tests showed that germ tube lengths were significantly shorter at 20‰ than 27‰ and at 27‰ than 35‰ ($p < 0.05$ for all comparisons with PG, Old, and New spores). The germ tube lengths of HC spores were not significantly different between 27‰ and 35‰ (Tukey; $p = 0.9999$).

3.3.4. – Light effects

Light intensity had a limited effect on *Nereocystis* spore development (Figures 3.5 and 3.6; Table 3.4 a and b). No significant effects were observed for sinking tendency, settlement success, and germination success of distant and Seldovia spores. Sinking tendency of germinated distant spores showed a significant relationship to light (ANOVA: $F = 3.72$; $p = 0.0371$), with sinking tendency indices being higher at 18 $\mu mol/m^2/s$ (3.5 ± 0.04) than 135 $\mu mol/m^2/s$ (3.3 ± 0.04) (Tukey; $p = 0.0286$ for comparisons between 18 versus 135 $\mu mol/m^2/s$; Figure 3.5 a). Light intensity was significant for germ tube length of all spores, regardless of their origin (PG or HC) or age (Old or New) (Figures 3.5 d and 3.6 d; Table 3.4 a and b). Light at 55$\mu mol/m^2/s$ had a significant negative impact on germ tube length compared to lower (0 and 18 $\mu mol/m^2/s$) and higher (135 $\mu mol/m^2/s$) light intensities (Tukey; $p < 0.05$ for comparisons between 55 versus 0, 18, and 135 $\mu mol/m^2/s$ for both distant and Seldovia spores).

3.3.5. – Origin effects

Spores collected from Port Graham and Halibut Cove displayed different sensitivities to salinity and light intensity regarding several parameters (Figures 3.3 and 3.5; Tables 3.3 a and 3.4 a). Sinking tendency of spores that already had germinated was significantly lower for PG than HC spores under both salinity and light treatments (Figures 3.3 a and 3.5 a; Table 3.3 a and 3.4 a). However, the Tukey test suggested that germinated PG and HC spores had similar sinking tendencies at low salinity (3.5 ± 0.1 and 3.6 ± 0.1, respectively; Tukey; $p = 0.9766$ at 20‰) and in darkness (3.5 ± 0.1 and 3.3 ± 0.1, respectively; Tukey; $p = 0.1008$ at 0 $\mu mol/m^2/s$). Under light treatments, settlement success of spores that had already germinated was significantly greater for PG than HC spores ($58.9 \pm 2.4\%$ versus $44.8 \pm 2.4\%$; Figure 3.5 b; Table 3.4 a). This pattern was significant at a higher level of significance ($\alpha = 0.1$) under salinity treatments, with $54.6 \pm 0.03\%$ versus $44.6 \pm 0.03\%$ of germinated PG and HC spores, respectively, that successfully settled (Figure 3.3 b; Table 3.3 a). Origin had a significant effect on germ tube length under both salinity and light treatments (Figures 3.3 d and 3.5 d; Tables 3.3 a and 3.4 a). Tukey tests showed that PG germ tube lengths were significantly shorter than those of HC under salinity (11.6 ± 0.3 µm versus 13.2 ± 0.3 µm; $p = 0.0043$) and light treatments (13.8 ± 0.2 µm versus 15.6 ± 0.2 µm; $p < 0.0001$).

3.3.6. – Age effects

Plant age had a significant effect on several parameters under both salinity and light treatments (Figures 3.4 and 3.6; Tables 3.3 b and 3.4 b). Sinking tendency of non-germinated Old spores was significantly greater than that of New spores under all salinity and light levels (3.6 ± 0.1 versus 2.5 ± 0.1 under salinity treatments, and 3.5 ± 0.1 versus 2.4 ± 0.1 under light treatments; Figures 3.4 a and 3.6 a). As mentioned previously, sinking tendency of non-germinated Old spores was greater than that of germinated Old spores, whereas sinking tendency of non-germinated New spores was lower than that of germinated

New spores (See section 3.3.2). Settlement success of non-germinated Old spores was significantly lower than that of New spores under both salinity and light treatments. On average, under salinity treatments, 21.9 ± 2.3% of non-germinated Old spores settled successfully versus 45.4 ± 2.3% of non-germinated New spores (Figure 3.4 b). Under light treatments, 25.7 ± 3.3% of non-germinated Old spores settled successfully versus 45.3 ± 3.3% of non-germinated New spores (Figure 3.6 b). Although a main age effect on settlement success of germinated Seldovia spores was noticed under light treatments (ANOVA; F = 5.27; $p = 0.0365$), Tukey tests revealed no significant differences between germinated Old and New spores and any light level ($p > 0.1$). Age was observed to have a significant effect on the germination success of Seldovia spores under both salinity and light treatments (Figures 3.4 c and 3.6 c). Old spores did not germinate as successfully as New spores under salinity treatments (78.7 ± 0.0% versus 86.8 ± 0.0%) and light treatments (77.6 ± 0.0% versus 87.7 ± 0.0%). Under both salinity and light treatments, germ tube lengths of Old and New spores were not significantly different (Figures 3.4 d and 3.6 d). On average, germ tube length of Old spores was 12.0 ± 0.3 µm versus 11.3 ± 0.3 µm for New spores under salinity treatments, and 14.0 ± 0.2 µm versus 14.0 ± 0.2 µm under light treatments.

3.4. – DISCUSSION

Salinity and light are critical physical factors influencing growth and survival, and consequently distribution, of many algal species. The successful development of spores, defined in this study as sinking tendency, settlement success, germination success, and germ tube length, is a necessary precursor to the subsequent establishment of gametophytic and sporophytic generations. This study suggests that low salinity has a potentially detrimental effect on the successful development of *Nereocystis* spores, with spores collected from an oceanic habitat and spores released from second-year plants being more sensitive than estuarine and New spores. Light was found to have a limited effect on *Nereocystis* spore development, regardless of origin or age.

3.4.1. – Salinity effects

Salinity is related to ion concentrations in and osmotic pressure of seawater, consequently exposure to changes in salinity can cause osmotic shock and have dramatic repercussions on algal physiology (Gessner and Schramm 1971; Reed *et al.* 1980; Russell 1987; Lobban and Harrison 1997; Kevekordes and Clayton 2000). Salinity can influence algal distribution and occurrence in estuaries (Scagel 1961; Norton and South 1969; den Hartog 1971; Kjeldsen and Phinney 1971) and can have a negative effect on spore germination, gametophyte development and fertilization, and embryo growth (Norton and South 1969; Druehl and Hsiao 1977; Young *et al.* 1987a, b; Anderson and Hunt 1988; Wright and Reed 1990; Anderson *et al.* 1992; Herbst and Castenholz 1994; Doblin and Clayton 1995; Burridge *et al.* 1999a,

b; Stekoll *et al.* 1999; Kevekordes 2000; Kevekordes and Clayton 2000). In this study, salinity was found to have a significant effect on several developmental parameters.

The spores of many algal species, including *Nereocystis*, possess flagella used for swimming that allow them to select a suitable settling location within the boundary layer (Amsler *et al.* 1992; Iken *et al.* 2000). Swimming competency may be affected by environmental factors but results from this study suggest that low salinity down to 20‰ did not have a significant impact on spore sinking tendency. Fucoid sperms change shape, swell up, and lose motility in response to osmotic shock caused by reduced osmotic pressure (0.25 OSmol/kg), and in extreme cases, sperm cells can lose their flagella (Wright and Reed 1990). No such developmental changes were observed in the present study.

Proper adhesion is fundamental for spore survival and successful recruitment of benthic macroalgal germlings (Neushul 1981b; Fletcher and Callow 1992; Vadas *et al.* 1992). In the present study, low salinity (20‰) inhibited settlement of germinated Seldovia spores suggesting that spores that had already germinated did not settle as securely under low salinity. Low salinity may have induced cellular damage that slowed the settling process, so that after 24 hours of incubation a lower proportion of spores settled than at higher salinity. Reduced salinity also may have impaired the adhesive secretion, consequently weakening the chemical bonding between spore and substrate. Reduced salinity (21‰) has a negative effect on the adhesion of *Hormosina banksii* embryos (Kevekordes 2000). Others have observed that reduced salinity can stunt development of rhizoids, which are involved in germinated spore and zygote attachment (Wright and Reed 1990; Anderson *et al.* 1992; Doblin and Clayton 1995; Kevekordes and Clayton 2000).

Germination rate also can be inhibited by low salinity (Wright and Reed 1990; Anderson *et al.* 1992; Doblin and Clayton 1995; Kevekordes and Clayton 2000). Osmotic shock and the resulting altered membrane permeability, which can limit cation uptake and disrupt intracellular electrical gradients, are thought to be the major cause of germination and growth inhibition (Burridge *et al.* 1996, 1999b). In this study, germination inhibition was observed only for distant spores at a salinity of 20‰, but not on Seldovia spores. Elongation of germ tubes was very sensitive to salinity. All spores, regardless of origin or age, showed an increase in germ tube length as salinity increased, with salinity effect most pronounced on oceanic spores. Cellular damage caused by osmotic shock or toxicants can impair chloroplasts, which are thought to be involved in germ tube elongation (Anderson and Hunt 1988; Kevekordes and Clayton 2000). The energy expended by spores trying to maintain an osmotic balance is energy that cannot be allocated to germination and growth.

In this study, high salinity was not observed to have a detrimental effect on any developmental parameter. A study on the effects of desalination plants on *Macrocystis pyrifera* spores also suggested that germination and germ tube length were not affected by a high salinity, 43‰ (Bay and Greenstein 2001). Osmotic potential of algal cells is more negative than that of seawater; therefore, salinity must increase

greatly before seawater becomes hypertonic compared to the inside of spores. In contrast, because seawater is already hypotonic with respect to spores, any reduction in salinity rapidly and dramatically increases spore strain (for review see Lobban and Harrison 1997).

3.4.2. - Light effects

Algal spore development can be affected by low and high irradiance levels (Amsler 1988a; Amsler and Neushul 1991; Reed *et al.* 1992; Graham 1996; Huovinen *et al.* 2000; Greer and Amsler 2002). In this study, sinking tendency, settlement success, and germination success were not significantly affected by light intensity. Spores of several algal species, including *Nereocystis*, can produce their own nutrition through photosynthesis (Kain 1964; Reed *et al.* 1988; Amsler and Neushul 1989, 1991; Reed *et al.* 1992; Brzezinski *et al.* 1993). In addition, *Macrocystis pyrifera* spores have internal carbon reserves of neutral lipids that can provide metabolic energy during dispersal for motility and germination (Brzezinski *et al.* 1993). Under photosynthetically saturating irradiance (85-140 $\mu mol/m^2/s$), *M. pyrifera* and *Pterygophora californica* spores can remain in suspension for up to 120 hours, whereas in the dark most spores stopped swimming after 48 hours, and none were seen swimming after 60 hours (Reed *et al.* 1992). In this study, no significant light effect was observed on sinking tendency; although *Nereocystis* spores were scored only 24 ±3 hours after spore release. In Reed *et al.* (1992), a significant effect of daylength on spore swimming capability did not occur until 48 hours after spore release. This suggests that spores in this study had sufficient internal carbon reserves to remain competent and motile 24 hours after spore release.

Light effects on spore attachment appear variable and specific to individual algal species. The adhesion of *Ceramium* sp. spores is light independent (Chamberlain 1976), whereas settlement densities of *Macrocystis pyrifera* and *Pterygophora californica* spores increase with daylength 24 hours after spore release (although variation occurred among individuals from different spore batches) (Reed *et al.* 1992). In contrast, the brown alga *Hincksia irregularis* was observed to have improved settlement success in the dark (Greer and Amsler 2002). *Hincksia irregularis* possesses an eyespot and displays negative phototactic behavior (directing it towards the substrate following release in the water column) (Greer and Amsler 2002), while most Laminariales, including *Nereocystis*, lack an eyespot and do not display phototaxis (Henry and Cole 1982). Several other studies using various kelp species also advise leaving spores in the dark for 30 minutes to 12 hours after release to increase spore settlement in the laboratory (Stekoll and Else 1990; Graham 1996; Amsler *et al.* 1999; Huovinen *et al.* 2000; Greer and Amsler 2002). In this study, no evidence of improved settlement success in the dark or under high irradiance was found after 24 hours of incubation. The lack of light effect on spore germination observed in this study was similar to results obtained by several authors (Lüning 1980; Anderson *et al.* 1990; Reed *et al.* 1992; Huovinen *et al.* 2000). As reported for *M. pyrifera* spores (Amsler 1988a, b; Brzezinski *et al.* 1993), *Nereocystis* spores may have sufficient stored energy to germinate within 24 hours in complete darkness.

In this investigation, light was found to have a significant effect on *Nereocystis* germ tube length but the relationship was not as expected. It is believed that germ tube length is a function of the spores' ability to perform photosynthesis. *Macrocystis pyrifera* spores grown under high light levels (100 $\mu mol/m^2/s$) produced longer germ tubes than spores grown at lower light intensities (50 $\mu mol/m^2/s$) (Anderson and Hunt 1988). Growth processes involving photosynthesis usually respond linearly to increasd irradiance until saturation is reached (for review see Ramus 1981). However, in this study, germ tube elongation was not different under darkness, 18 $\mu mol/m^2/s$, or 135 $\mu mol/m^2/s$, but was significantly shorter under 55 $\mu mol/m^2/s$. This pattern is intriguing since the range of light intensities used in this study is commonly used for algal culture (Bolton and Lüning 1982; Hoffman and Santelices 1982; Deysher and Dean 1984; Reed *et al.* 1991, 1992; Greer and Amsler 2002). The saturating irradiance for *Nereocystis* spores is only 40-50 $\mu mol/m^2/s$ (Amsler 1988a); maximum germ tube elongation was therefore expected at 55 $\mu mol/m^2/s$. The pattern of germ tube growth observed in this study may suggest that there is a range of light intensities where spores do not grow as well as at higher or lower light levels. It is tentatively speculated that *Nereocystis* spores consume stored photosynthate or maternal carbon reserves to continue growing in the dark or at very low light level (0 and 18 $\mu mol/m^2/s$). In contrast, at intermediate light levels (55 $\mu mol/m^2/s$), spores might receive too much light to undertake the light-independent carbon fixation process but not enough light to assimilate carbon through photosynthesis as efficiently as under higher light levels. *Nereocystis* spores also may allocate their energy towards processes other than germ tube elongation when incubated under 55 $\mu mol/m^2/s$.

Effects of other factors (temperature or salinity) also may vary in response to experimental light levels. The interaction between irradiance and temperature has been noticed in several studies on both propagules and sporophytes (e.g. Lüning and Neushul 1978; Lüning 1980; Bolton and Lüning 1982; Bolton and Levitt 1985; Deysher and Dean 1986b; tom Dieck 1993; Stekoll *et al.* 1999). As example, irradiance levels required for gametogenesis of *Laminaria* sp. spores increased exponentially as temperature increased (Lüning 1980). Because temperature was used as a covariate and not a factor in the statistical model, it was not possible to investigate the interaction effect of light*temperature or salinity*temperature in this study.

3.4.3. - Origin effects

Different populations of the same species may respond differently to environmental conditions because of phenotypic plasticity (den Hartog 1971; Geesink 1973; Gerard and Mann 1979) or genotypic variation (Russell and Bolton 1975; Bolton 1979; Reed and Russell 1979; Gerard *et al.* 1987; Young *et al.* 1987a), or a combination of both (Yarish *et al.* 1979). Thus, caution should be taken against conducting experiments using populations from very different environments without considering origin as a source of variation because of the potential presence of ecotypes that have different genotypes (Russell and Fielding 1981). No differences in appearance were observed under the microscope between oceanic and estuarine

spores in this study. However, spores collected from those two distant sites seemed to respond differently under various salinity and light conditions. Spores collected from the estuarine environment were not significantly affected by salinity or light (except for germ tube length). In contrast, spores collected from the oceanic site were more sensitive to low salinity. The sinking tendency of germinated estuarine spores was greater than that of oceanic spores at higher salinity and in light treatments, whereas there was no difference in the sinking tendency of non-germinated spores between the two sites. Reduced and elevated salinities can induce a change in volume and ionic content of spores (Reed *et al.* 1980; Wright and Reed 1990), resulting in a modification of spore buoyancy and subsequent sinking rate (Coon *et al.* 1971; Clayton 1992). If spores from an oceanic environment were neutrally buoyant at high salinity, they would become negatively buoyant at lower salinities and sink faster. In contrast, if spores from an estuarine environment were neutrally buoyant at low salinity, they would become positively buoyant at higher salinities and sink less. Because the expected pattern was not observed, a simple buoyancy process cannot explain the different sinking tendencies between oceanic and estuarine spores so other factors or processes (such as flagellar beating, mucus secretion) must be involved.

A greater proportion of oceanic spores that germinated were securely attached to the substrate compared to germinated estuarine spores. As such, the estuarine spores probably started germinating in the water column or were faster at reaching the substrate and germinating than oceanic spores, but they did not settle as securely. It is generally believed that spores first settle, attach, and then germinate (Amsler *et al.* 1992; Clayton 1992; Fletcher and Callow 1992; Pillai *et al.* 1992). However, *Laminaria hyperborea* spores have been observed to start germinating while in the water column without impairing their subsequent attachment (Kain 1964). *Pterygophora californica* spores also can maintain their adhesion ability when germinating prior to settling, while *Macrocystis pyrifera* spores lose their ability to successfully settle and attach after germinating (Reed *et al.* 1992).

The combination of successful settlement and germination is critical to the subsequent production of gametophytes. Despite the effect of origin on sinking tendency and settlement success of germinated distant spores, germination success 24 hours after release was similar for oceanic and estuarine spores. The sequence of developmental steps may differ between estuarine and oceanic spores, with estuarine spores possibly germinating before settling. Germ tube length of all spores was hampered by low salinity and was shorter at a light intensity of 55 $\mu mol/m^2/s$, although oceanic spores were usually significantly shorter than estuarine spores (except at higher salinity). This investigation suggests an origin effect on several spore developmental parameters. However, it is difficult to know if the observed differences between oceanic and estuarine spores are due to the presence of genetically distinct ecotypes or phenotypes, as no data are available to assess whether Kachemak Bay kelp beds are genetically different.

3.4.4. - Age effects

Nereocystis is considered an annual because it produces only one stipe during its lifetime and cannot grow new tissue once the upper stipe is destroyed (Nicholson 1970). However, because individuals that mature late in the season may survive until the next spring, *Nereocystis* may be regarded as a "facultative biennial" (Foreman 1970). In Kachemak Bay, many *Nereocystis* plants overwinter to grow new blades and become reproductive their second season. It is possible that second-year plants are important for kelp bed survival. To contribute substantially to successful recruitment of new cohorts, the spores produced by older plants have to develop successfully. No differences were observed under the microscope in the visual appearance of Seldovia Old versus New spores. Both age classes looked healthy and seemed to have similar swimming behaviors and patterns; however, significant differences were observed between Old and New spores for several developmental parameters.

Twenty-four hours after spore release, a greater number of Old, non-germinated spores was found within the boundary layer than New, non-germinated spores. In addition, most Old spores that had sunk had not germinated whereas most New spores within the boundary layer had germinated. These observations suggest that Old spores did not swim as long and did not germinate as fast as New spores. This is supported by the observation that, under both salinity and light treatments, more non-germinated Old spores were found within the boundary layer than germinated Old spores. For New spores, the trend was reversed; a greater number of germinated than non-germinated spores was found within the boundary layer. As a result, it is thought that non-germinated New spores may retain their swimming capability longer than Old spores. This may allow spores produced by first-year plants to better select a suitable settling location and to ultimately be more successful at recruiting than spores produced by second-year plants.

Settlement success of germinated spores was similar for both age classes. Once germinated, similar proportions of Old and New spores resisted the washing event and successfully settled. However, a greater proportion of non-germinated Old spores had not settled 24 hours after spore release compared to New spores, indicating that Old spores were slower at attaching to the substrate or were less adhesive compared to New spores. Since spore attachment improves over time as chemical bonds form between spore and substrate (Charters *et al.* 1971, 1973; Coon *et al.* 1971), 24 hours may not have been enough time to reach their maximum adhesion strength. In addition, the lower germination success of Old spores suggests that Old spores are slower at germinating than New spores. Differences in germination rates can be critical in establishing the dominance of competing spores. In the Baltic Sea, the dominance of adult *Pilayella littoralis* over *Enteromorpha* spp. is caused by differences in germination rates of microscopic stages (Reed 2000).

Although, no significant differences were observed for germ tube growth between Old and New spores, Old spores may not develop as successfully as New spores. The lower developmental success of Old spores compared to New spores is not based on their inability to successfully settle and germinate but

is caused by the slower rate at which they settled and germinated. Once they settle and germinate, Old spores grow germ tubes as well as New spores. However, spores have high mortality rates, and the slower they develop, the longer they are exposed to hazards (Vadas *et al.* 1992). Because of competition for space and resources, spores that can settle and germinate fast may have an advantage over slower spores. Therefore, over the course of a season, spores produced by first-year plants are thought to contribute more to recruitment than spores produced by second-year plants.

3.5. – CONCLUSION

This investigation suggests that estuarine conditions can affect *Nereocystis* spore fitness but the salinity and light levels used in this study were not extreme enough to be lethal to spores. Results may have been more dramatic if salinity and light intensity levels similar to waters closer to the head of the bay had been used. Although salinity effects were not strong enough to kill spores, osmotic stress may increase spore susceptibility to other detrimental factors.

Nereocystis spores from different sites responded differently under salinity and light treatments, suggesting that they may be adapted or acclimated to conditions at their original site. It is currently unclear whether spores from different kelp beds are genetically different or demonstrate phenotypic plasticity. It also was found that although second-year plants become reproductive during their second season, their spores do not develop as fast as first-year spores. The contribution of second- year plants to the recruitment of new *Nereocystis* generations may be limited during the second growing season.

Although information on spore sensitivity is critical to a better understanding of kelp bed dynamics, there is a gap between laboratory work and *in-situ* results. Limitations in extrapolating laboratory results to the field exist, and many questions about the actual meaning of spore development, as defined by sinking tendency, settlement success, germination success, and germ tube length, remain unanswered. In this study, germ tube length was more sensitive than other developmental parameters to salinity and light. Although the ecological consequence of longer versus shorter germ tubes is unclear, germ tube length is commonly used as an endpoint for pollution bioassays because of its high sensitivity to pollutants and variations in environmental conditions.

This study was a "snap shot" of what happens at 24 ± 3 hours after spore release. It provides useful information on potential factors influencing *Nereocystis* spore development in estuaries. However, additional laboratory work on other microscopic stages and on shorter and longer-term exposures is required to further understand the spatial distribution and temporal variation of estuarine kelp beds. Other studies should focus on the effects of low salinity if spores are immersed for only short periods, such as at low tide when *Nereocystis* blades and sori are temporarily in a freshwater layer. It is presently unknown whether spores that are released within this freshwater layer remain viable and can successfully develop

when they reach the substrate. Additional work on the effects of light intensity is also required to elucidate the response that *Nereocystis* spores displayed at 55 µm mol/m^2/s in this experiment.

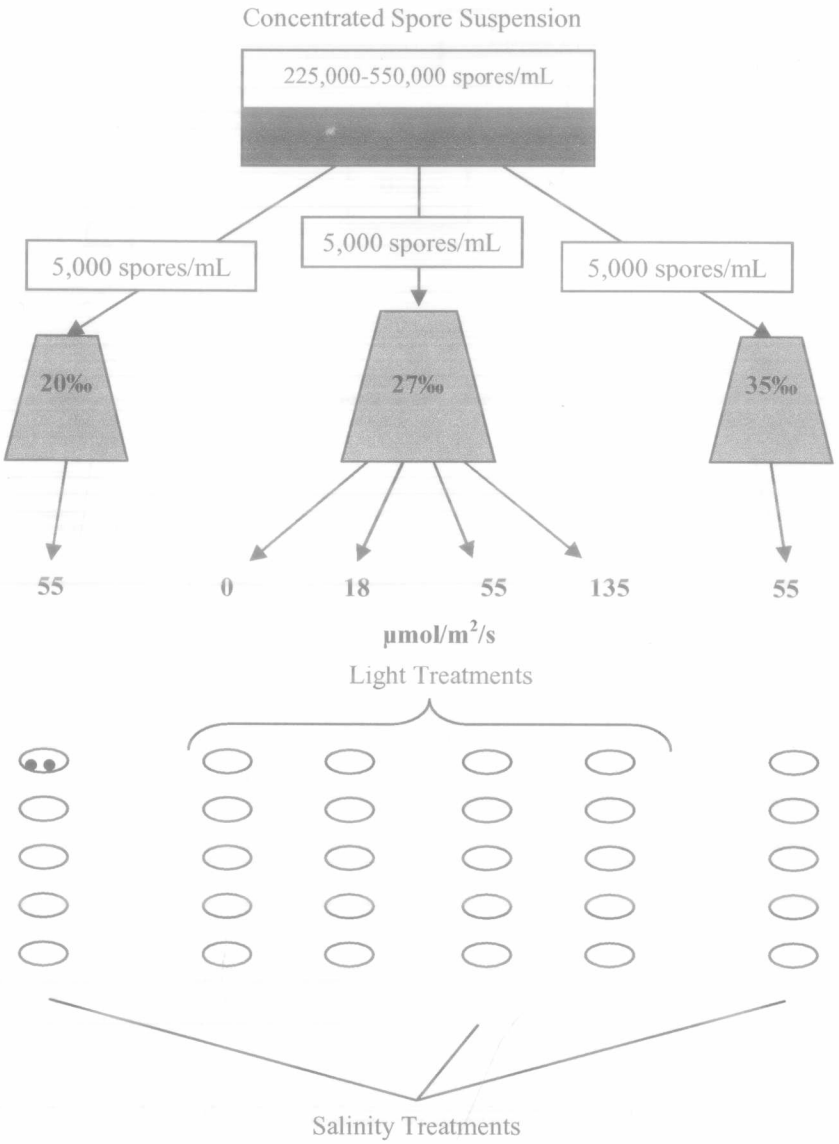

Figure 3.1: Diagram representing the combination of three salinity (20, 27, 35‰) and four light (0, 18, 55, 135 µmol/m^2/s) levels used for the different treatments. Salinity treatments were grown under 55 µmol/m^2/s and light treatments were grown in seawater at 27‰. The set of petri dishes combining a salinity of 27‰ and light level of 55 µmol/m^2/s was shared by both salinity and light treatments. Each treatment had five replicates (petri dishes) containing two cover slips; a total of thirty petri dishes were scored per experiment. Each petri dish had a final concentration of 5,000 spores/mL in suspension.

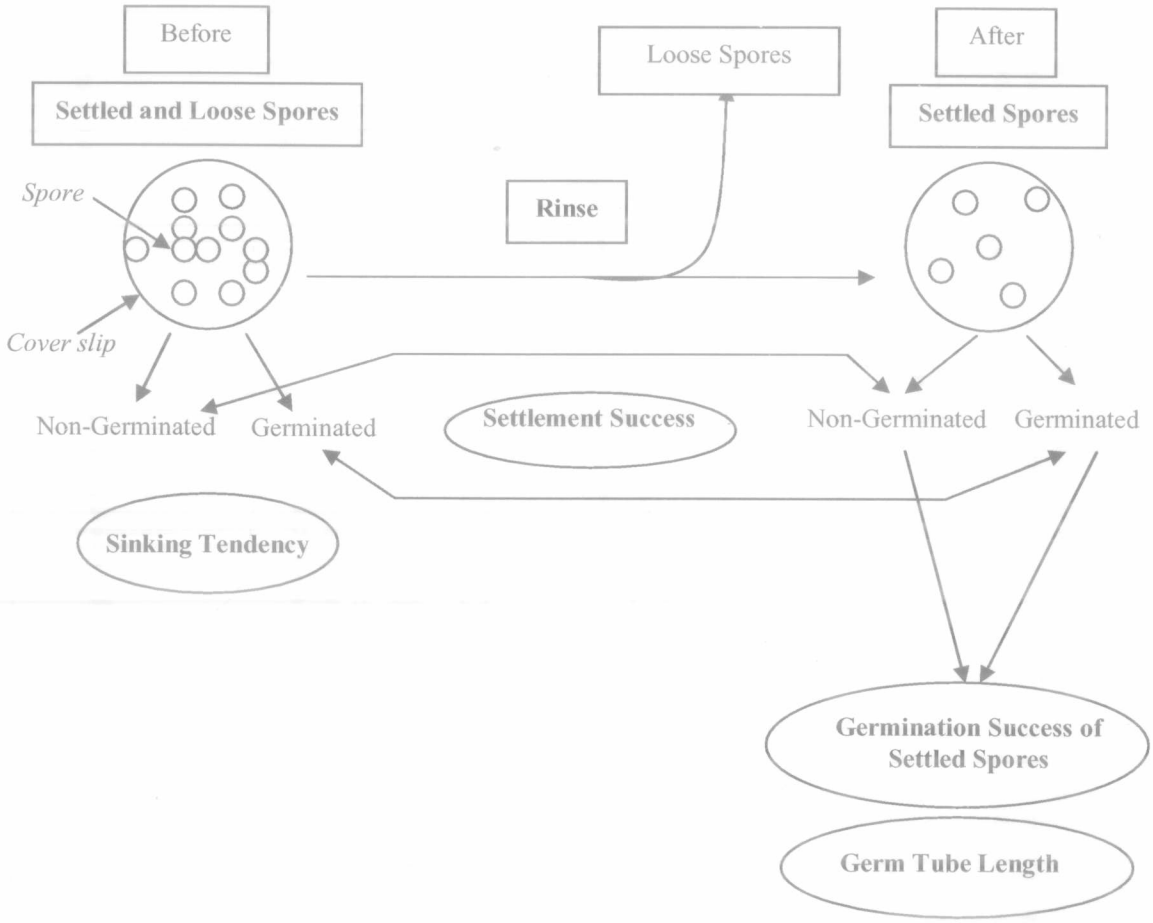

Figure 3.2: Flowchart representing the different parameters studied and the scoring procedure steps. Sinking tendency of non-germinated and germinated spores was estimated by counting the number of spores within five fields of view before rinsing the cover slip. Settlement success was estimated for non-germinated and germinated spores by comparing the numbers before and after rinsing. Germination success was calculated as a percentage of germinated spores in reference to the total number of adhered spores (after rinsing). Germ tube length was measured on ten settled spores.

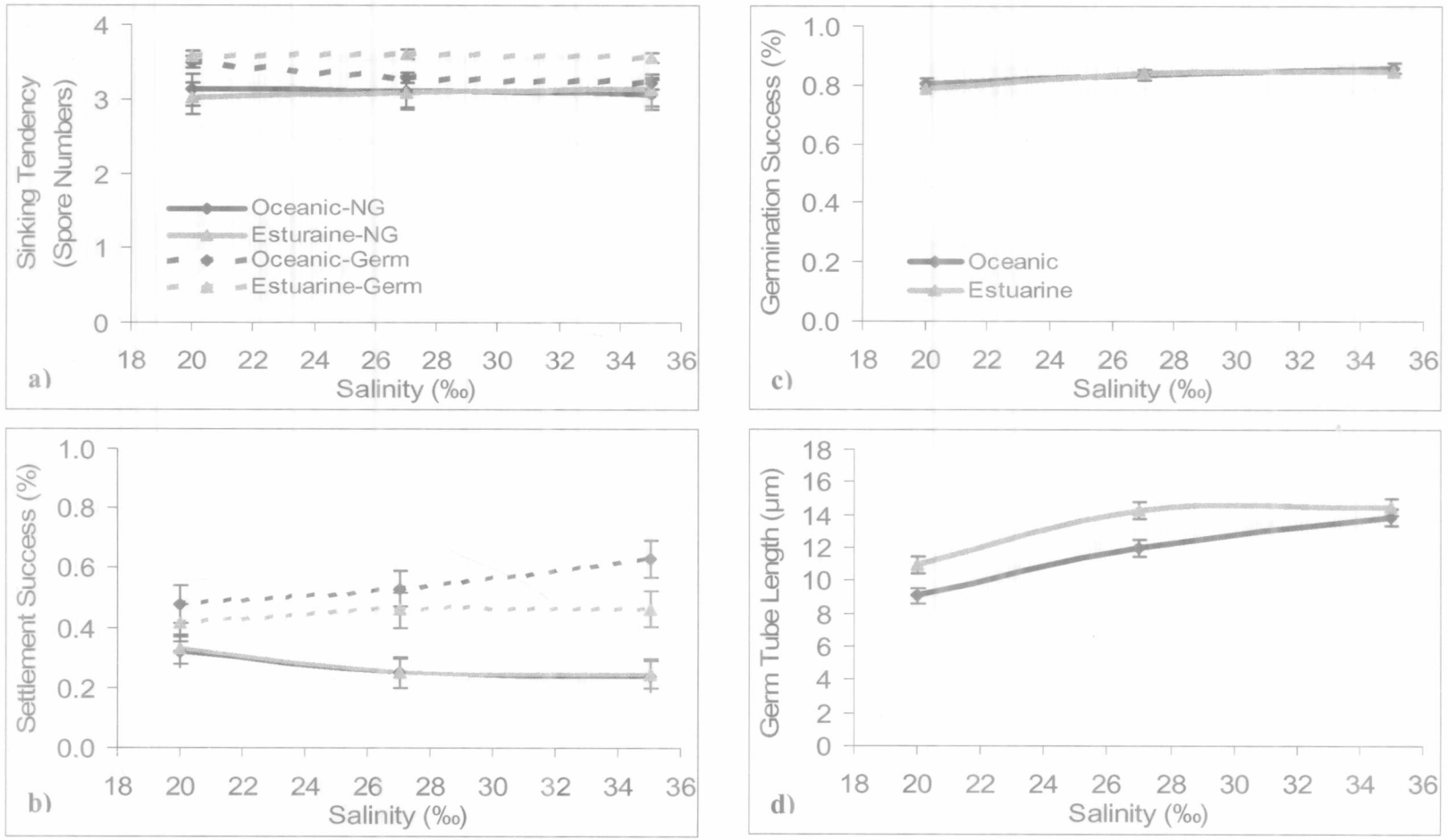

Figure 3.3: Effects of salinity (20, 27, 35‰) and origin (Oceanic from Port Graham or Estuarine from Halibut Cove) on: a) sinking tendency, b) settlement success, c) germination success, and d) germ tube length. The data for sinking tendency, settlement success, and germination success were arcsine, square-root transformed. The values represents LS means (± 1 SE; estimated by the Satterthwaite Approximation) based on scores from five petri dishes per experiment x three experiments (n = 3). Sinking tendency and settlement success were observed separately for non-germinated (NG) and germinated (Germ) spores. Germination success and germ tube length were observed on settled spores only.

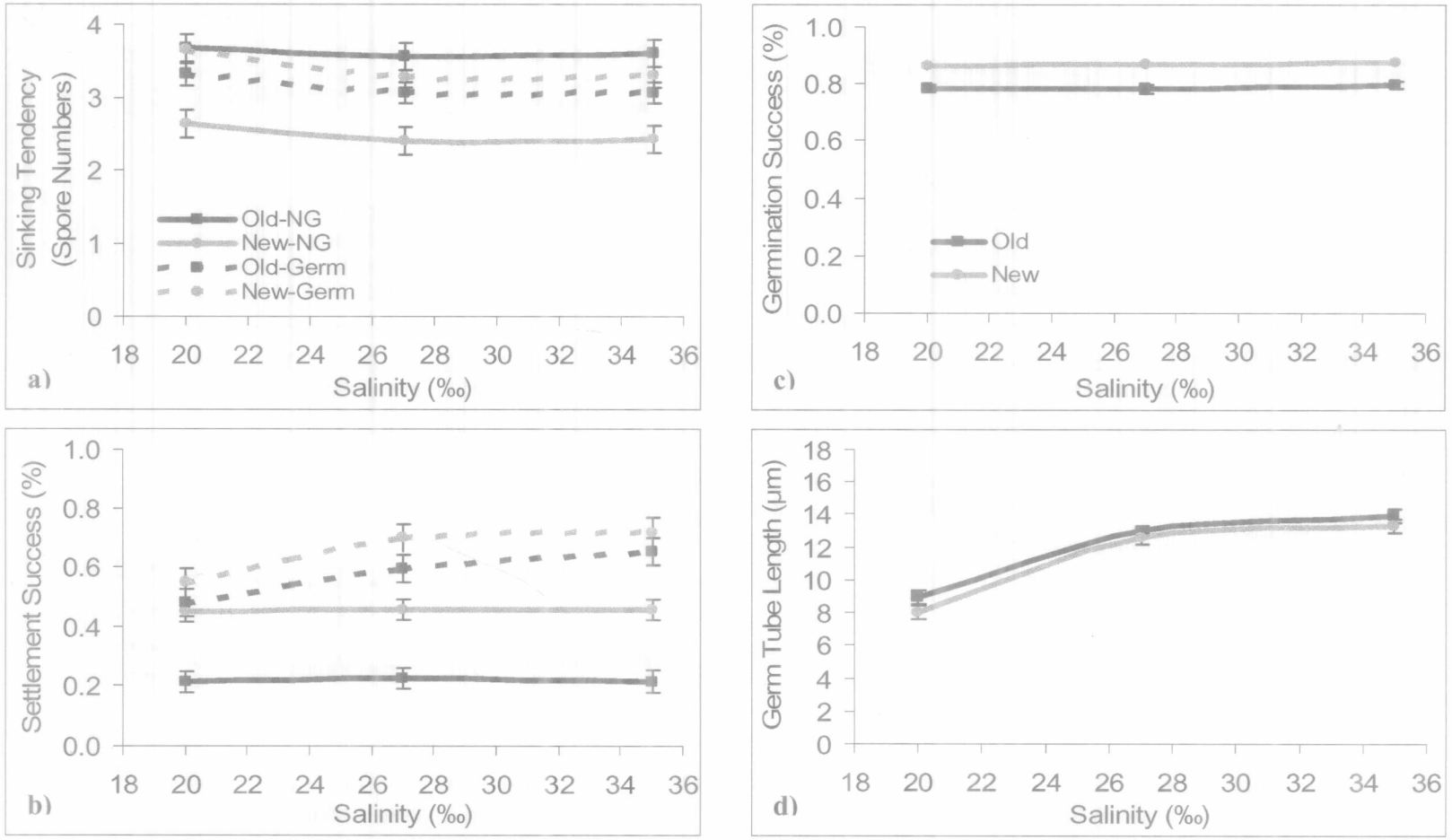

Figure 3.4: Effects of salinity (20, 27, 35‰) and age (Old and New) on: a) sinking tendency, b) settlement success, c) germination success, and d) germ tube length. The data for sinking tendency, settlement success, and germination success were arcsine, square-root transformed. The values represents LS means (± 1 SE; estimated by the Satterthwaite Approximation) based on scores from five petri dishes per experiment x three experiments (n = 3). Sinking tendency and settlement success were observed separately for non-germinated (NG) and germinated (Germ) spores. Germination success and germ tube length were observed on settled spores only.

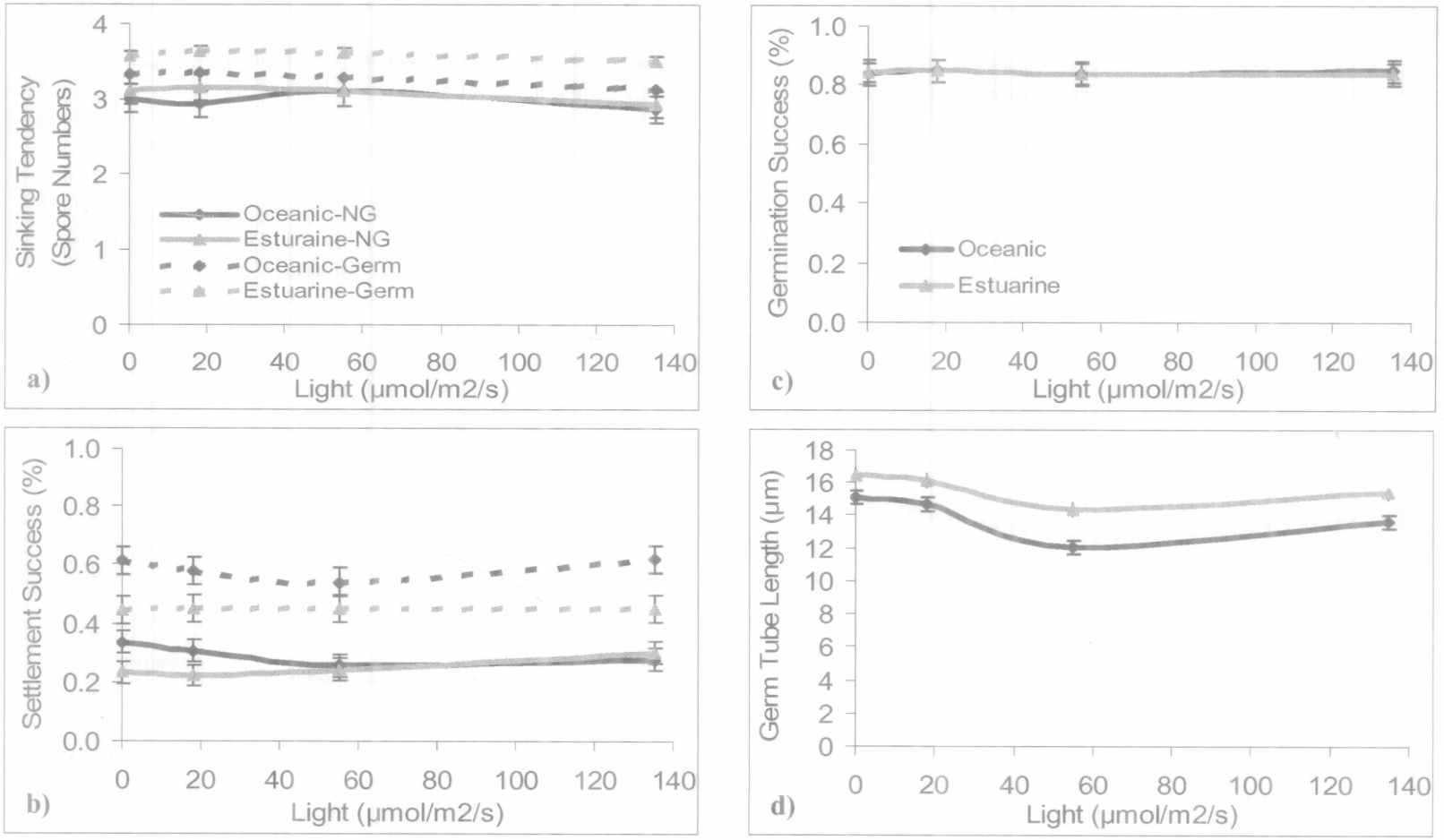

Figure 3.5: Effects of light (0, 18, 55, 135 µmol/m^2/s) and origin (Oceanic from Port Graham or Estuarine from Halibut Cove) on: a) sinking tendency, b) settlement success, c) germination success, and d) germ tube length. The data for sinking tendency, settlement success, and germination success were arcsine, square-root transformed. The values LS means (± 1 SE; estimated by the Satterthwaite Approximation) based on scores from five petri dishes per experiment x three experiments (n = 3). Sinking tendency and settlement success were observed separately for non-germinated (NG) and germinated (Germ) spores. Germination success and germ tube length were observed on settled spores only.

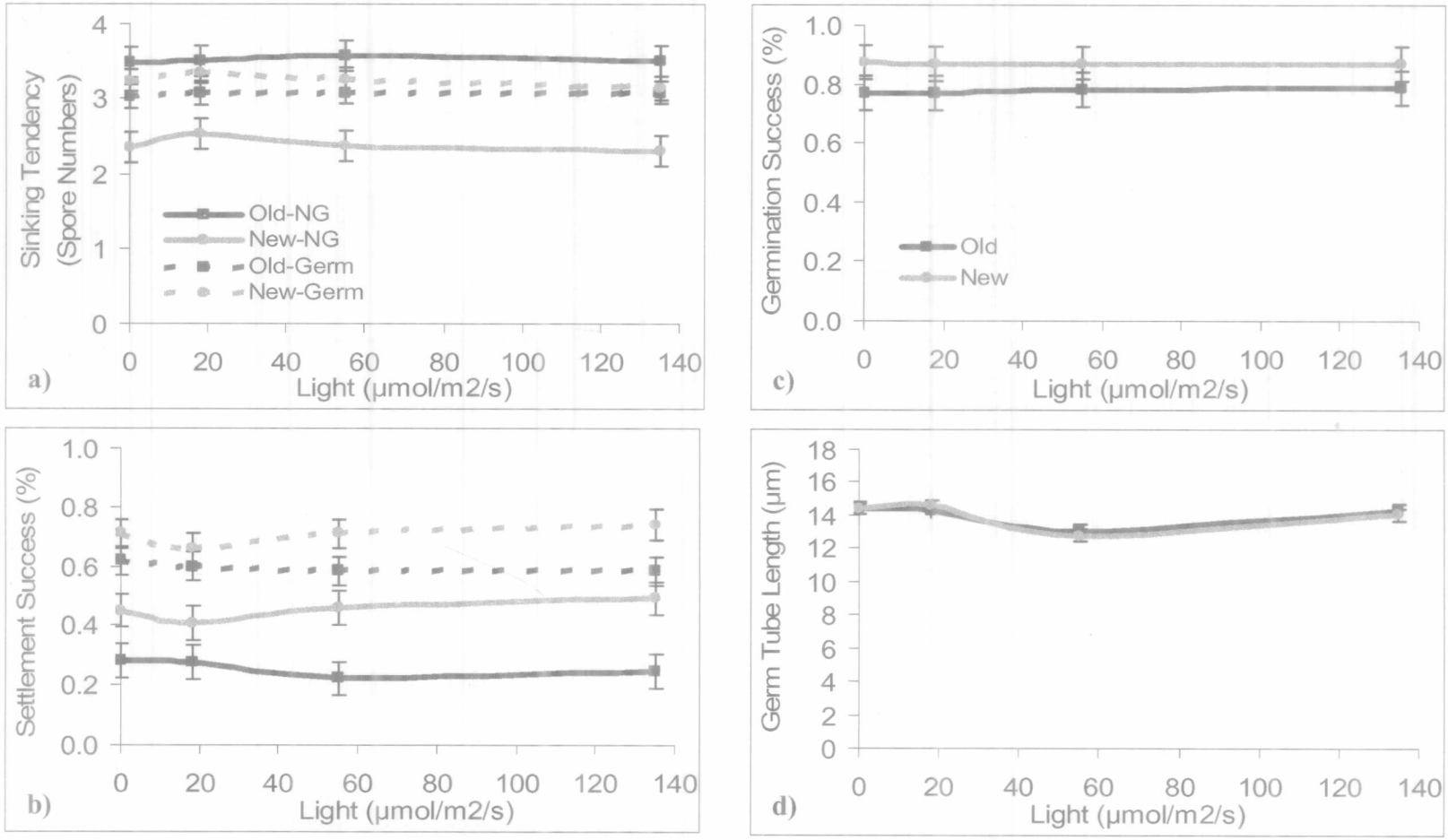

Figure 3.6: Effects of light (0, 18, 55, 135 µmol/m^2/s) and age (Old and New) on: a) sinking tendency, b) settlement success, c) germination success, and d) germ tube length. The data for sinking tendency, settlement success, and germination success were arcsine, square-root transformed. The values represents LS means (± 1 SE; estimated by the Satterthwaite Approximation) based on scores from five petri dishes per experiment x three experiments (n = 3). Sinking tendency and settlement success were observed separately for non-germinated (NG) and germinated (Germ) spores. Germination success and germ tube length were observed on settled spores only.

Table 3.1: Summary statistics on incubation temperatures (° C) for each experiment, based on temperature measurements made every ten minutes. Three experiments were conducted for each of the following spore categories: Port Graham (PG), Halibut Cove (HC), Seldovia Old (Old), Seldovia New (New). n = 132 to 140 depending on the exact incubation time.

	PG 1	**PG 2**	**PG 3**	**HC 1**	**HC 2**	**HC 3**
Mean	**11.04**	**10.83**	**12.59**	**11.55**	**11.69**	**12.86**
S. E.	0.07	0.06	0.03	0.06	0.06	0.06
Range	3.51	3.51	1.55	3.49	3.87	4.25
Minimum	9.42	9.42	11.77	10.21	10.60	10.60
Maximum	12.93	12.93	13.32	13.70	14.47	14.85

	Old 1	**Old 2**	**Old 3**	**New 1**	**New 2**	**New 3**
Mean	**12.81**	**10.12**	**12.93**	**9.42**	**9.00**	**10.88**
S. E.	0.10	0.08	0.04	0.04	0.04	0.06
Range	3.88	2.75	2.31	3.53	2.37	2.72
Minimum	10.21	8.63	12.16	8.63	8.23	10.21
Maximum	14.09	11.38	14.47	12.16	10.60	12.93

Table 3.2: Pearson correlation coefficients (r) for relationships between mean incubation temperatures or times and the different parameters studied (sinking tendency of non-germinated spores, SinkNG; sinking tendency of germinated spores, SinkGerm; settlement success of non-germinated spores, SSNG; settlement success of germinated spores, SSGerm; germination success of settled spores, GSSett; germ tube length, GTL). Values are in parentheses if $p > 0.05$. n = 360 (five petri dishes/treatment x six treatments/experiment x twelve experiments).

	Pearson Coefficients	
Parameters	Mean Temperature	Time
SinkNG	0.46	(0.06)
SinkGerm	(0.04)	(0.05)
SSNG	-0.57	(-0.09)
SSGerm	-0.29	(-0.06)
GSSett	(0.03)	(0.07)
GTL	0.30	0.28

Table 3.3: Results of analyses of variance on effects of: a) salinity (20, 27, 35‰) and origin (Port Graham or Halibut Cove) and b) salinity and age (Old or New) on *Nereocystis* spores. The response variables are sinking tendency of non-germinated and germinated spores (SinkNG and SinkGerm, respectively), settlement success of non-germinated and germinated spores (SSNG and SSGerm, respectively), germination success of settled spores (GSSett), and germ tube length (GTL). (DF numerator degree of freedom, F statistics and P value estimated by Satterthwaite Approximation). Values significant at the $\alpha = 0.05$ level are marked by *.

a)

		SinkNG		SinkGerm		SSNG	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	0.56	0.4703	9.51	0.0104*	2.17	0.1693
Time	1	1.70	0.1963	0.81	0.3706	0.10	0.7480
Origin	1	0.02	0.8962	15.67	0.0022*	0.01	0.9078
Salinity	2	0.01	0.9890	2.26	0.1502	1.62	0.2413
Origin*Salinity	2	0.08	0.9700	2.24	0.1526	0.01	0.9905

		SSGerm		GSSett		GTL	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	0.79	0.3941	0.94	0.3546	3.80	0.0779
Time	1	0.01	0.9137	1.31	0.2557	52.54	<0.0001*
Origin	1	3.66	0.0826	0.27	0.6152	13.03	0.0043*
Salinity	2	1.42	0.2836	4.68	0.0346*	36.63	<0.0001*
Origin*Salinity	2	0.49	0.6267	0.16	0.8576	1.47	0.2729

b)

		SinkNG		SinkGerm		SSNG	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	28.87	0.0002*	2.37	0.1516	124.79	<0.0001*
Time	1	3.26	0.0787	1.50	0.2294	0.50	0.4804
Age	1	30.96	0.0002*	2.47	0.1439	37.97	<0.0001*
Salinity	2	0.50	0.6178	3.29	0.0760	0.02	0.9824
Age*Salinity	2	0.08	0.9219	0.10	0.9023	0.01	0.9937

		SSGerm		GSSett		GTL	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	3.65	0.0826	0.11	0.7490	44.42	<0.0001*
Time	1	1.50	0.2242	1.87	0.1763	112.05	<0.0001*
Age	1	2.87	0.1174	30.55	0.0002*	1.25	0.2876
Salinity	2	8.70	0.0054*	0.46	0.6422	116.09	<0.0001*
Age*Salinity	2	0.13	0.8754	0.06	0.9381	0.32	0.7293

Table 3.4: Results of analyses of variance on effects of: a) light (0, 18, 55, 135 µmol/m^2/s) and origin (Port Graham or Halibut Cove) and b) light and age (Old and New) on *Nereocystis* spores. The response variables are sinking tendency of non-germinated and germinated spores (SinkNG and SinkGerm, respectively), settlement success of non-germinated and germinated spores (SSNG and SSGerm, respectively), germination success of settled spores (GSSett), and germ tube length (GTL). (DF numerator degree of freedom, F statistics and P value estimated by Satterthwaite Approximation). Values significant at the $\alpha = 0.05$ level are marked by *.

a)		SinkNG		SinkGerm		SSNG	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	0.55	0.4710	20.15	0.0005*	2.07	0.1709
Time	1	1.03	0.3125	0.33	0.5646	1.17	0.2829
Origin	1	0.53	0.4768	57.24	<0.0001*	2.37	0.1443
Light	3	0.47	0.7077	3.72	0.0371*	0.49	0.6939
Origin*Light	3	0.13	0.9402	0.42	0.7407	1.22	0.3379

		SSGerm		GSSett		GTL	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	0.03	0.8671	4.45	0.0527	7.96	0.0131*
Time	1	0.08	0.7774	0.46	0.4989	39.47	<0.0001*
Origin	1	15.70	0.0013*	0.00	0.9757	31.86	<0.0001*
Light	3	0.29	0.8337	0.33	0.8028	15.97	<0.0001*
Origin*Light	3	0.33	0.8051	0.50	0.6878	0.51	0.6801

b)		SinkNG		SinkGerm		SSNG	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	26.98	0.0001*	2.52	0.1332	45.18	<0.0001*
Time	1	0.06	0.8014	0.43	0.5143	0.39	0.5344
Age	1	34.48	<0.0001*	1.60	0.2214	13.10	0.0025*
Light	3	0.17	0.9156	0.19	0.9014	0.16	0.9213
Age*Light	3	0.16	0.9215	0.21	0.8852	0.52	0.6779

		SSGerm		GSSett		GTL	
Source of variation	DF	F	P	F	P	F	P
Mean Temperature	1	4.44	0.0525*	1.18	0.2949	26.63	0.0001*
Time	1	0.09	0.7662	0.03	0.8641	60.10	<0.0001*
Age	1	5.27	0.0365*	57.3	<0.0001*	0.02	0.9031
Light	3	0.21	0.8862	0.15	0.9251	8.54	0.0015*
Age*Light	3	0.38	0.7717	0.43	0.7338	0.34	0.7988

Table 3.5: Results of a t-test on paired observations on the sinking tendency and settlement success of non-germinated (NG) versus germinated (Germ) *Nereocystis* spores. The comparison is made for distant spore (Port Graham and Halibut Cove) and Seldovia spores (Old and New) under both salinity (20, 27, 35‰) and light (0, 18, 55, 135 µmol/m^2/s) treatments. The set of perti dishes exposed to 27‰ and 55 µmol/m^2/s (Control) was shared by both salinity and light treatments. The difference between the two groups represents the mean difference (df = 14). n = 15 (five petri dishes/per treatment x three experiments for each spore category).

		SINKING TENDENCY (NG – Germ)			SETTLEMENT SUCCESS (NG – Germ)		
Origin	Treatment	t-value	p-value	Difference	t-value	p-value	Difference
Port Graham	20‰	-5.29	0.0001	-0.382	-4.71	0.0003	-0.149
	Control	-1.99	0.0661	-0.187	-10.72	<0.0001	-0.271
	35‰	-1.18	0.2575	-0.157	-9.70	<0.0001	-0.383
Halibut Cove	20‰	-21.07	<0.0001	-0.553	-2.49	0.0262	-0.097
	Control	-18.53	<0.0001	-0.511	-4.79	0.0003	-0.222
	35‰	-6.41	<0.0001	-0.419	-10.23	<0.0001	-0.237
Port Graham	0 µmol/m^2/s	-2.80	0.0142	-0.335	-7.61	<0.0001	-0.262
	18 µmol/m^2/s	-3.79	0.0020	-0.417	-8.60	<0.0001	-0.260
	135µmol/m^2/s	-2.29	0.0379	-0.260	-11.24	<0.0001	-0.332
Halibut Cove	0 µmol/m^2/s	-16.66	<0.0001	-0.451	-8.42	<0.0001	-0.226
	18 µmol/m^2/s	-18.49	<0.0001	-0.496	-6.89	<0.0001	-0.239
	135µmol/m^2/s	-13.57	<0.0001	-0.659	-5.45	<0.0001	-0.164
Old	20‰	3.07	0.0083	0.339	-8.24	<0.0001	-0.306
	Control	4.64	0.0004	0.472	-12.94	<0.0001	-0.406
	35‰	4.29	0.0007	0.496	-16.76	<0.0001	-0.466
New	20‰	-24.90	<0.0001	-0.982	-2.51	0.0250	-0.063
	Control	-11.43	<0.0001	-0.850	-5.85	<0.0001	-0.208
	35‰	-14.28	<0.0001	-0.884	-5.58	<0.0001	-0.226
Old	0 µmol/m^2/s	3.65	0.0027	0.432	-11.12	<0.0001	-0.381
	18 µmol/m^2/s	4.04	0.0012	0.405	-19.61	<0.0001	-0.366
	135µmol/m^2/s	3.58	0.0030	0.389	-15.89	<0.0001	-0.380
New	0 µmol/m^2/s	-14.43	<0.0001	-0.861	-8.86	<0.0001	-0.216
	18 µmol/m^2/s	-19.54	<0.0001	-0.794	-7.68	<0.0001	-0.209
	135µmol/m^2/s	-10.43	<0.0001	-0.803	-4.70	0.0003	-0.205

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CHAPTER 4

EFFECTS OF HERBIVORY BY *LACUNA VINCTA* ON *NEREOCYSTIS LUETKEANA* IN KACHEMAK BAY, ALASKA

4.1. - INTRODUCTION

The dynamics of kelp communities results from complex interactions between various biotic and abiotic factors. Herbivory is a major biological factor that influences kelp bed structure (Leighton 1971; Duggins 1980; Lubchenco and Gaines 1981; Dayton 1985; Vadas 1985). Herbivory can dramatically alter the fitness, growth, and survival of individual algae (Steinberg 1984; Johnson and Mann 1986) as well as influence plant abundance, distribution, diversity, and succession at the population and community levels (Lubchenco and Gaines 1981). Many organisms, including echinoderms, polychaetes, crustaceans, mollusks, and fishes graze on benthic algae (for review see, North 1971; Foster and Schiel 1985). In temperate and high latitude algal communities, sea urchins, snails, and amphipods are the major grazers (Vadas 1985).

Many studies have described the role of sea urchins in kelp forest dynamics (e.g. Mann and Breen 1972; Palmisano and Estes 1976; Harrold and Reed 1985; Estes *et al.* 1998; Konar 2000a, b). As a dominant species, sea urchins can influence and shape the entire community structure by limiting algal abundance and diversity or in extreme cases by overgrazing the majority of the algal flora in response to a change in feeding behavior from feeding on drifts to actively foraging on live kelp (Leighton 1971; Mann 1973; Foster 1975; Pearse and Hines 1979; Dean *et al.* 1984; Harrold and Reed 1985). They are a crucial link in the balance between healthy kelp beds and barren grounds (e.g. Estes and Palmisano 1974; North and Pearse 1974; Lawrence 1975; Duggins 1980; Harris *et al.* 1984; Harrold and Reed 1985; Vadas 1985; Dean *et al.* 1989; Steinberg 1995; Leinaas and Christie 1996). The green urchin *Strongylocentrotus droebachiensis* has been found in densities up to 57 individuals/m^2 in Kachemak Bay, Alaska and is thought to have contributed to the disappearance of the entire kelp bed on the Archimandritof Shoals in 2002 (Figure 1.2) (Chenelot *et al.* 2001).

While sea urchins are well-known kelp grazers, forty other invertebrate grazers (echinoderms, mollusks, and arthropods) have been found to inhabit kelp beds (Foster and Schiel 1985). Whereas macrograzers such as urchins damage older thalli by decreasing photosynthetic tissue, shortening the life span, or limiting development and growth, cryptic micrograzers such as amphipods mostly impact the survival of microalgae and microscopic life stages (Vadas 1985). Many mollusks (e.g. *Tegula brunnea. T. funebralsi*, and *Notoacmea insessa*) that graze on the surfaces of kelp blades and stipes can cause severe tissue weakening but rarely cause the amputation of the full thalli. Some organisms (e.g. *Calliostoma*

annulatum, *C. ligatum*, and *Mitrella carinata*) mainly feed on bryozoans, hydroids, diatoms, or detritus found on kelp surfaces and are thought to have a minimal impact on kelp survival (Leighton 1971). In contrast, mesograzers such as *Lacuna unifasciata* and *Idotea resecata* produce holes in stipes and blades (Foster and Schiel 1985), which may become centers of infection and indirectly jeopardize plant health (Jones 1971). Kelp holdfasts also harbor a great variety of micro-organisms (Ghelardi 1971) such as the isopod *Limnoria algarum*, wich can burrow through haptera causing the base of the holdfast to weaken and the plant to become dislodged (Jones 1971).

Lacuna vincta is a small, herbivore commonly found in the low intertidal to subtidal zone of the northeastern Pacific (Fralick *et al.* 1974; Johnson and Mann 1986; Martel 1991; Martel and Chia 1991a, b; Martel and Diefenbach 1993; Padilla 2001). It is rarely observed on benthic substrata (Padilla 2001) and uses kelp for habitat and food (Martel 1991; Padilla 1998; Toth and Pavia 2002a). In British Columbia, it is the dominant species found in *Macrocystis integrifolia* and *Nereocystis luetkeana* canopies (Martel and Chia 1991a). In the San Juan Archipelago, *L. vincta* was observed to represent 94% of the grazers found on *Nereocystis* (Duggins *et al.* 2001). Most studies on the feeding preference of grazers were conducted with either *L. vincta* feeding on kelp species other than *Nereocystis* (Johnson and Mann 1986; Chavanich and Harris 2002) or were on the palatability of *Nereocystis* with grazers other than *L. vincta* (Steinberg 1985; Morris and Campbell 1996; Van Alstyne *et al.* 1999, 2001; Pelletreau and Muller Parker 2002). Little is known about the interaction between *L. vincta* and *Nereocystis*.

The need for a grazing effect and feeding preference study with *L. vincta* on *Nereocystis* of different age classes became apparent during a transplant experiment studying *Nereocystis in-situ* growth rates in Kachemak Bay, Alaska (See Chapter 2). Aware of the potential danger of urchin grazing, the kelp transplants were raised above the substrate; however, this did not preclude *L. vincta* settlement. This chapter reports on transplant survivorship throughout the summer (2001) and snail abundance on the transplants in September 2001. The feeding preference of *L. vincta* on *Nereocystis* blades of different age classes was also investigated in the laboratory.

4.2. – METHODS

4.2.1. – *In-situ* kelp

The three study sites (Port Graham, Seldovia, and Halibut Cove) are located on the south side and along the axis of Kachemak Bay, Alaska (described in Chapters 2 and 3; Figure 1.2). Although the sites are characterized by different salinity and turbidity conditions, they have similar current regimes. Port Graham, located at the entrance of the bay, is characterized by oceanic conditions whereas Halibut Cove is more estuarine. Seldovia is located 20 km inward of Port Graham and 30 km outward from Halibut Cove.

During the summer of 2001, only two urchins were encountered in the study area. However, great numbers of mesogastropods were observed on *Nereocystis* as well as other kelp species. The dominant species on *Nereocystis* was the lacunid snail *Lacuna vincta*. The archeogastropods *Tegula funebralis*, *Calliostoma ligatum*, and *Margarites pupillus* were occasionally observed on *Nereocystis*, but in smaller abundances (personal observation). *Margarites pupillus* resided primarily on *Agarum clathratum* but very rarely on *Nereocystis* (Shawn Harper, personal communication).

Reciprocal transplants of *Nereocystis* sporophytes were completed amongst all study sites in early July 2001. A total of 36 healthy juveniles were collected from each study site. Out of those 36 individuals, 12 were transplanted to each of the three sites (including their site of origin). The transplants were attached at a depth of 8 m MLLW to a ground line that was kept off the seafloor to prevent urchin grazing (see Chapter 2 for more details).

Transplant survival was monitored weekly using scuba from July 16 to August 16, 2001 and then again on September 10. Survivorship was calculated as the percent of individuals alive at the time of the census in reference to the number alive on July 16. To avoid including handling casualties in the counts, only individuals that survived the first week after being transplanted were included in the survey. An ANOVA for repeated measures followed by a Tukey test was performed to determine differences in plant survival at each site and from each origin.

The abundance of *L.vincta* snails at each study site was recorded from individual transplants on September 10, 2001. Because of the great number of snails encountered and the difficulty of thoroughly surveying individual blades underwater, only snails found on stipes and pneumatocysts were counted. Plants that had lost their blades due to snail grazing also were included in snail counts. The mean number of snails per transplant at each site was calculated and the difference between sites was investigated using a one-way ANOVA followed by a pairwise Tukey test.

4.2.2. - Laboratory grazing experiments

Based on observations made during regular visits to the study sites, it appeared that *L. vincta* snails were found on both juvenile and adult *Nereocystis*, but juveniles showed more grazing impact (personal observation). Laboratory experiments were conducted to investigate *L. vincta* preference for juvenile rather than adult and old blades as food and substrate. For this study, juvenile refers to individuals that were less than approximately 1 m in length, adult refers to larger individuals that are less than 1 year old, and old refers to individuals that are in their second growing season.

Grazing experiments were conducted in September 2001 at the Kasitsna Bay Laboratory using an outdoor flow-through seawater system. *Nereocystis* blades of different age classes and *L. vincta* snails were collected at the Seldovia site the day before the experiment. The blades were gently brushed to remove epiphytes. Eight blades from each age class were cut in pieces of 50 cm^2 (5 by 10 cm for old and adult or

2.5 by 20 cm for juvenile blades) and weighed. The weight of each piece was approximately 5.5 ± 0.2 g (SE), 3.1 ± 0.1 g, and 2.7 ± 0.1 g for old, adult, and juvenile blades, respectively. The difference in weight was due to blades of different age classes having different thicknesses (0.99 ± 0.02 cm, 0.45 ± 0.02 cm, and 0.34 ± 0.03 cm, respectively).

A combination of treatments was chosen to determine whether *L. vincta* preferred juvenile, adult, or old *Nereocystis* blades. Twenty-five snails were placed in a plastic chamber (27 x 30 x 12 cm) containing either juvenile (J), adult (A), or old blades (O). In other chambers, snails were given a choice of two blade types (J-O, J-A, or Y-O) or all three blade types simultaneously. Each treatment was performed once for 48 hours. To assess ontogenic changes during the experiment, two control containers with all three blade types but with no snails also were monitored. The snails were stirred gently after being introduced to the containers to randomize their settlement.

To determine feeding preference of *L. vincta*, the weight change of each blade type was compared. Each blade was blotted dry and weighed prior to the start and at the termination of the experiment. The change in weight for each blade was calculated by subtracting the initial from the final weight. To account for any weight change not due to herbivory, the mean weight change of the control blades of each age class was subtracted from the mean weight change calculated for each age class. Because the different treatments were not replicated, no statistics were performed. However, because trends in weight loss of each age class were similar under all treatments, the total mean-weight change for each age class was calculated by pooling together blades from all treatments (n = 4).

To determine the extent of grazing damage on different age class blades, the percentage of each blade's total surface area damaged by grazing was estimated. The difference between grazed and ungrazed tissue was obvious as the translucent medulla was exposed by grazing and contrasted clearly against the darker cortex layer. A grid was used and random squares, each representing 4% of the blade's total surface area (50 cm^2), were scored for grazing marks. Since the trends in surface area grazed for each age class were similar under all treatments, the total mean surface area grazed for each age class was calculated by pooling together blades from all treatments (n = 4).

To determine whether *L. vincta* preferentially resided on one type of blade, the location of the snails (on juvenile, adult, or old blades, or on the container wall) was recorded for each chamber several times over the 48-hour period (4, 8, 19, 25, 32, 45, and 48 hours after the start of the experiment). The results are presented as the total number of snails found on each substrate type (old, adult, and juvenile blades, or walls) from all containers together. No statistics were performed on these data.

4.3. – RESULTS

4.3.1. – *In-situ* observations

Nereocystis survivorship at all three sites was relatively high and constant over the first six weeks of the *in-situ* experiment (Figure 4.1). Up to mid-August, 93.6 ± 3.3%, 88.1 ± 8.1%, and 94.4 ± 5.6% of the plants transplanted in Port Graham, Seldovia, and Halibut Cove, respectively had survived. Three weeks later a severe decline in survivorship, especially in Seldovia, was observed. On September 10, only two plants (or 5.6 ± 5.6%) had survived in Seldovia. Survivorship also decreased at the other two sites, but not in similar proportions. On September 10, 57.4 ± 4.9% and 48.3 ± 11.1% of the transplants survived in Port Graham and Halibut Cove, respectively. A one-way ANOVA for repeated measures showed that there was no significant difference in transplant survivorship over the first five weeks of the experiment ($p > 0.1$ for all five dates). However, survivorship was significantly lower at Seldovia than at the other two sites on September 10 (repeated measures ANOVA; $F = 60.50$; $p = 0.0010$; n = 3).

The role of the site of origin on transplant survival was found to be significant on 2 census dates. By August 6, 87.7 ± 3.0%, 97.3 ± 2.7%, and 97.3 ± 2.7% of the transplants originating from Port Graham and Seldovia and Halibut Cove had respectively survived (ANOVA for repeated measures; $F = 17.33$; $p = 0.0107$; n = 3). On August 15, there was no significant difference in transplant survival based on their origin (repeated measures ANOVA; $F = 6.50$; $p = 0.0554$; n = 3). At the last census, on September 10, more transplants originating from Seldovia had survived (51.3 ± 17.2%) than transplants collected from Port Graham (32.67 ± 16.8%) and Halibut Cove (27.7 ± 14.7%) (repeated measures ANOVA; $F = 12.25$; $p = 0.0197$; n = 3).

Tiny *Lacuna vincta* snails appeared in mid July on the kelp at Seldovia and Port Graham but not at Halibut Cove. Their numbers and size increased over the following weeks and very soon obvious thalli damage of *Nereocystis* was visible, both amongst the transplants and the surrounding kelp beds (personal observation). In September, snail density was significantly different between the study sites (ANOVA; $F = 26.81$; $p < 0.0001$; n = 23 for Port Graham, n = 8 for Seldovia, n = 24 for Halibut Cove). In Halibut Cove, out of the eighteen surviving plants, snails inhabited four plants, with a mean density of 0.17 ± 0.1 snails/plant. Out of 23 surviving plants in Port Graham, the mean density was 2.8 ± 0.7 snails/plant, with a range of 12 to 0 snails for individual plants. In contrast, only two plants survived in Seldovia but an additional six nonviable transplants (that had lost their blades and had a punctured pneumatocyst) were used in snail counts. The mean density was 74.4 ± 25.0 snails/plant with a range of 210 to 0 snails for individual plants.

4.3.2. – Laboratory grazing experiments

In all feeding treatments, *L. vincta* preferentially fed on juvenile blades while old blades were the least favored (Figure 4.2). The weight loss for each age class blade in the single treatment was 0.04 g. 0.29

g, and 0.36 g for old, adult, and juvenile blades, respectively. A similar trend was observed when the snails were offered all three blade types simultaneously: old blades lost 0.04 g to grazing, adult blades lost 0.10 g, but juvenile blades lost 0.53 g. When the snails had the choice between two blade types, old blades had the least weight loss (0.01 g and 0.16 g when offered with adult and juvenile blades, respectively) whereas juvenile blades had the greatest weight loss (0.43 g and 0.34 g when offered with old and adult blades, respectively). Adult blades lost 0.18 g when offered with old blades and 0.25 g when offered with juvenile blades. When the mean total weight loss (based on all treatments) was compared between all blade types, juvenile blades lost the greatest amount (0.42 ± 0.04 g) whereas old blades lost the least amount (0.06 ± 0.03 g) and adult blades lost on average 0.21 ± 0.04 g.

Herbivory pressure was examined by comparing surface areas with radula marks between the different blade age classes (Figure 4.3). The radula marks left by the snails were quite obvious as exposed clear medulla. In many instances, juvenile blades were completely punctured whereas only the superficial outer medulla layer of old blades was grazed (personal observation). The proportion of surface area grazed by *L. vincta* for blades of different age classes when only one blade type was available was 46%, 62%, and 67% for old, adult, and juvenile blades, respectively. A similar trend was observed when the snails were offered all three blade types simultaneously, with the surface areas grazed being 41%, 36%, and 78% for old, adult, and juvenile blades, respectively. When the snails had a choice between two blade types, the smallest surface area grazed was found in old blades (32% and 39% when offered with adult and juvenile blades, respectively), whereas the largest surface area grazed was for juvenile blades (57% and 47% when offered with old and adult blades, respectively). The percent surface area grazed for adult blades was 41% when offered with old blades and 50% when offered with juvenile blades. Similar trends to that observed for weight loss were seen in the overall proportions of grazed surface areas for specific age classes. All treatments combined, on average, 62.1 ± 6.6% of the juvenile blades were grazed whereas only 39.7% ± 5.0% and 47.3 ± 5.8% of the old and adult blades, respectively, were grazed.

When *L. vincta* snails were given the choice of substrates, either *Nereocystis* blades or the container walls, they preferentially settled on blades rather than walls (although a greater surface area of walls was available compared to that of blades) (Figure 4.4). During the experiment, between 59% (after 19 hours) and 83% (after 48 hours) of the snails were counted on blades instead of walls. The total number of snails (based on all containers) on juvenile blades varied between 64 and 75 throughout the experiment whereas it ranged between 28 and 54 on old blades and 24 and 48 on adult blades. Although *L. vincta* snails were seen more often on juvenile blades than on old and adult blades, the data do not clearly show a strong substrate preference. Some snail movement from one substrate to another was observed as suggested by the fluctuation in snail numbers for each substrate over time. If a strong preference for juvenile blades as substrate was present, the number of snails found on juvenile blades would be expected to have increased over time, while it would have decreased for other substrate types.

4.4. DISCUSSION

Grazing plays an important role in the regulation of algal communities. Sea urchins are commonly thought to be the top grazer in kelp communities. In extreme cases they can overgraze a kelp bed to barren ground when their densities are unchecked (Mann and Breen 1972; Duggins 1980; Estes *et al.* 1998) or when they switch their foraging behavior from a sit-and-wait strategy feeding on kelp drift to a more active strategy feeding on attached kelp thalli (Ebeling *et al.* 1985; Harrold and Reed 1985; Konar 2000a). Other kelp grazers, such as gastropods, isopods, and fishes, also have been reported to have a critical effect on algal communities (Jones 1971; Leighton 1971; North 1979; Harris *et al.* 1984; Foster and Schiel 1985; Vadas 1985; Padilla 1993; Leonard 1994; Van Alstyne *et al.* 1999; Toth and Pavia 2002a, b).

In Kachemak Bay, grazing by *Lacuna vincta* on *Nereocystis* transplants was significant and promoted high mortality by destroying stipes and blades. *L. vincta* density patterns among the three study sites seemed to coincide with *Nereocystis* survivorship. In Seldovia, where snail densities reached 210 snails per transplant, survivorship dropped to 5.6 ± 5.6% by the end of the summer, whereas survivorship remained around 50% in Port Graham and Halibut Cove, where snail densities were significantly lower. This observation suggests that *L. vincta* can play a key role in kelp bed dynamics. In contrast with grazers such as limpets and chitons, or gastropods such as *Margarites* and *Calliostoma* spp. that eat primarily diatoms, *L. vincta* successfully grazes on macroscopic sporophytes and is thought to be an important factor in structuring subtidal communities (Fralick *et al.* 1974; Johnson and Mann 1986). Grazers typically directly consume less than 20% (and often less than 10%) of the algal biomass; most algal biomass is believed to go through the detrital food web (Vadas 1985). In one study, only 0.05% of the total annual blade biomass (fresh weight) of *Laminaria longicruris* was directly consumed by *L. vincta* (Johnson and Mann 1986). Although this amount is negligible and did not cause plant mortality, the actual impact of herbivory on the kelp canopy was significant as grazing was concentrated on blades that can be easily shredded and torn away and not on stipes and holdfasts. In contrast, another study reported extensive damage and mortality of *Laminaria saccharina* and *L. digitata* as a result of intense *L. vincta* grazing (Fralick *et al.* 1974). In the present study, many of the young plants, either transplanted or in the surrounding kelp beds, had lost their blades and most of the meristoderm layer of their thalli to grazing (personal observation). Some plants were in critical shape as their pneumatocyst had lost its flotation due to grazing holes. Punctures of the pneumatocysts result in plant death (Foreman 1970). Most dead plants in this study had only the holdfast remaining with stipes broken off at the base where stipe diameter is minimal. The decrease in survivorship in Port Graham and Halibut Cove between mid-August and mid-September was not as pronounced as in Seldovia but was more prominent than between mid-July and mid-August. Toward the end of the summer, warmer water temperatures may have increased transplant stress making them more vulnerable to even moderate grazing pressures and to other impairments such as parasites or lower salinities. Additionally, in Halibut Cove, the anchor line damaged two transplants.

Nereocystis stipes are adapted to survive strong currents, however if the stipe is damaged, it loses its tensile strength and easily breaks (Koehl and Wainwright 1977; Denny *et al.* 1997). It has been shown that *L. vincta* grazing makes kelp more vulnerable to physical forces such as currents (Duggins *et al.* 2001). The three study sites in this paper displayed, on average, moderate current and wave exposure characteristics. In Kachemak Bay, tidal cycles are extreme (with a mean range of 4.7 m and a maximum of 8.5 m) and produce strong cyclic tidal currents. The current regimes at the study sites may fit into the "lethal" category described by Duggins *et al* (2001) and may enhance the destructive effect of mesograzers. The magnitude and oscillatory current patterns at the study sites are suspected to be periodically slow enough to allow snails to invade the thalli and cause severe damage, while at other times to be strong enough to exceed the breaking strength of damaged thalli. Also, the considerable dispersal ability of *L. vincta* (during its post-metamorphic stage) (Smith 1973; Fretter and Manly 1977; Martel 1991; Martel and Chia 1991a, b) gives this gastropod a high capacity of colonization (Johnson and Mann 1986). Juvenile and adult *L. vincta* have the ability to "sail" through the water column with the current by extending their foot and antenna and producing mucus threads that allow them to travel easily between plants and to be transported long distances through the water column (Johnson and Mann 1986; Martel 1991; Martel and Chia 1991a, b; Martel and Diefenbach 1993). A potential contributing factor to the limited presence and influence of *L. vincta* in Halibut Cove may be that the kelp bed at that site was small and therefore transplants were outside (approximately 10 to 50 m away) the kelp bed. In contrast, in Port Graham and Seldovia, the transplants were within larger kelp beds surrounded by infested plants. An alternate explanation maybe that fewer snails successfully dispersed that far up the bay from the outer bay, as Halibut Cove is several kilometers away from potential source beds.

In Kachemak Bay, kelp growth was temporally variable at each of the study sites. The plants emerged earliest in Port Graham, then in Seldovia, and last in Halibut Cove. In Port Graham, much of the *Nereocystis* kelp was close to the surface and juvenile plants (50 cm in length) were scarce when the *in-situ* experiment was started in early July. In Seldovia, the *Nereocystis* was not as large as in Port Graham and small plants were more abundant. In Halibut Cove, most of the *Nereocystis* plants were juveniles. It is therefore suggested that most of the plants in Port Graham had already reached a refuge size when herbivory pressure was strong whereas in Seldovia the timing of kelp and snail recruitment seemed to be more synchronous. In Seldovia, many plants were still small and tender when the snails were grazing. It has been proposed that, in response to grazing threats, *Nereocystis* apportions most of its resources to growth instead of chemical defense in order to rapidly reach a refuge size (Steinberg 1985; Van Alstyne *et al.* 2001). *Nereocystis* does have one of the fastest growth rates of any plants, up to 25 cm per day when combining stipe and blade growth (Nicholson 1970). In Kachemak Bay, the stipe of transplanted *Nereocystis* grew as much as 14.7 cm/day (see Chapter 2). From incidental field observations, *L. vincta*

appeared to be most abundant on juvenile plants and juvenile plants appeared to be more damaged by grazing than older plants. The results from the laboratory grazing study mirrored these field observations.

Like most brown algae, *Nereocystis* produces polyphenolic compounds as an antigrazing mechanism (Steinberg 1984, 1985; Johnson and Mann 1986), however, the levels found in this species are low and not thought to be effective at deterring grazers (Steinberg 1984, 1985; Johnson and Mann 1986). In a preference feeding study of the snail *Tegula funebralis*, *Nereocystis*, like all other algae preferred by *T. funebralis*, had low phenolic contents (only 0.44% of algal dry mass) and low relative astringency compared to the least preferred algae, which had high phenolics (up to 4.93% dry mass for *Pelvetiopsis limitata*) and high tannin equivalents (above 5.5% dry mass) (Steinberg 1985). *Nereocystis* exhibits an unusual trend in phlorotannin concentrations as a function of plant length in juveniles with concentration increasing with plant length, with juvenile tissues containing 43% less phlorotannin than adult tissues (Van Alstyne *et al.* 2001).

In addition to chemical defenses, other anti-herbivory plant characteristics can include nutritional quality (Johnson and Mann 1986; Chavanich and Harris 2002), tissue toughness (Padilla 1985; Steinberg 1985; Johnson and Mann 1986; Van Alstyne *et al.* 2001; Chavanich and Harris 2002) and thallus morphology (Steneck and Watling 1982; Johnson and Mann 1986; Chavanich and Harris 2002). Nutritional quality can play a substantial role in grazing selection (Steinberg 1985; Johnson and Mann 1986; Van Alstyne *et al.* 2001; Chavanich and Harris 2002). However, nutritional values are similar in juvenile and adult *Nereocystis*, with nitrogen content < 3% of dry mass and carbon to nitrogen ratios < 15 (Steinberg 1985; Van Alstyne *et al.* 2001). Thallus toughness was important in *Tegula* food preference amongst chemically undefended algae (like *Nereocystis*) but was not found to affect grazer preference on algal species with high phenol content (Steinberg 1985). Tissue toughness influences the ability of mesograzers like *L. vincta* that possesses a taenioglossan radula because it is not very effective at chewing through leathery tissue (Steneck and Watling 1982).

Tissues of juvenile algae are thinner and easier to puncture than tissues of older individuals (Van Alstyne *et al.* 1999, 2001). The present study showed that a greater proportion of juvenile blades surface area was damaged compared to older blades. In addition, juvenile blades were observed being completely punctured and becoming almost translucent due to extensive grazing damage. This may be related to older blades being thicker than adult and juvenile blades (0.99 cm versus 0.45 and 0.34 cm, respectively). When grazing damages were observed under a microscope, radula marks appeared to be equally deep on juvenile and old blades (personal observation). This may suggest that the meristoderm of old blades is an effective 'barrier' against grazing. Once snails puncture the tough outer layer on older blades, they seem able to graze similarly on the cortex and medulla of all blades. It is therefore probable that older blades are more difficult to consume than younger blades and may explain the choice of *L. vincta* to graze on softer juvenile tissue.

4.5. - CONCLUSION

The results from the present study and anecdotal field observations suggest that *Lacuna vincta* can have dramatic impacts on *Nereocystis* populations. High *Nereocystis* mortality coincided with sites of high grazing pressure. Grazing by *L. vincta* was also observed to have a greater effect on juvenile than adult and old *Nereocystis* blades. Replication of the feeding experiments and additional work on feeding benefits versus anti-grazing mechanisms (chemical defenses and tissue toughness) of juvenile versus adult plants are desired to better understand the grazing behavior and impact of *L. vincta* on *Nereocystis*.

Grazing activities were found to be patchy and therefore not believed to be a major factor in the large-scale distribution of *Nereocystis* kelp beds. At a local scale, further ecological investigations may help determine if grazing pressure can be sufficient to limit the number of plants reaching maturity, and in turn reducing the number of spores produced and the recruitment of the subsequent *Nereocystis* generation. Since juvenile kelps are more susceptible to grazing than older plants, the synchrony in recruitment timing of grazers and algae also seems critical and worthy of further investigation. Spatial and temporal variability in the occurrence of *L. vincta* (Johnson and Mann 1986) and that of *Nereocystis* (Schoch 2001) has been reported, however, little is known about the dynamics between those two species.

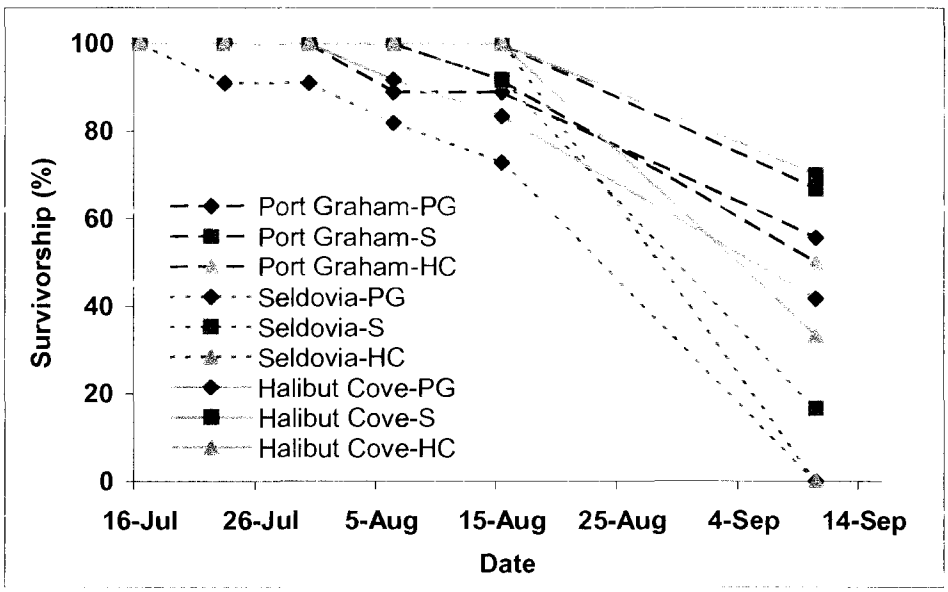

Figure 4.1: Survivorship of *Nereocystis* transplants of different origins at each study site during Summer 2001. Survivorship is calculated as the percent of individuals alive at the time of the census in reference to the number of individuals alive one week after the transplants. The study sites are represented by dashed lines (Port Graham), dotted lines (Seldovia), or full lines (Halibut Cove). The origins are represented by diamonds (PG), squares (S), or triangles (HC).

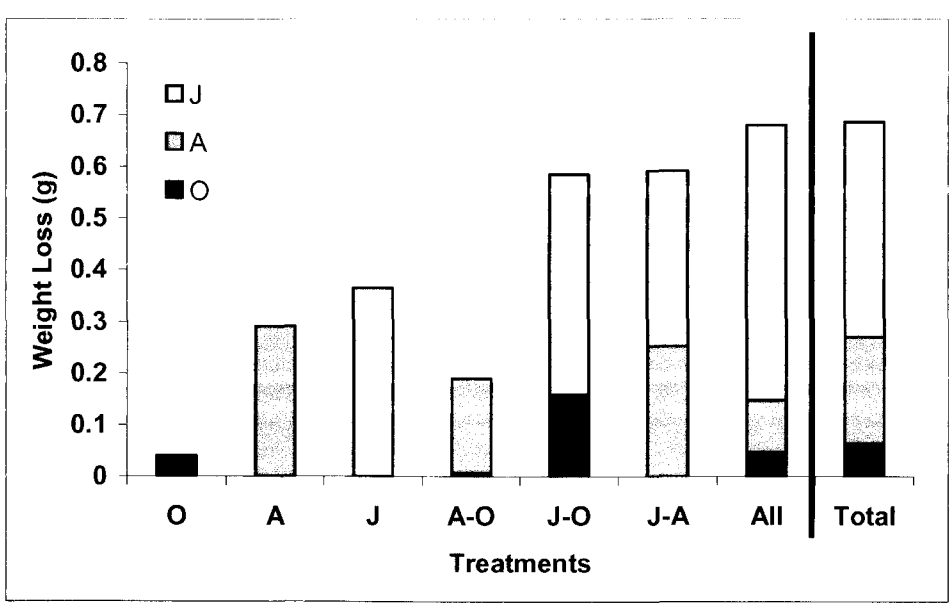

Figure 4.2: Change in weight for each blade age-class after 48 hours of grazing pressure by *Lacuna vincta*. Snails were offered either old (O), adult (A), or juvenile blades (J) separately, had the choice between O and A blades, O and J blades, or A and J blades, and were also offered all three blade types simultaneously (All) (n = 1 for each treatment). 'Total' represents the weight loss for each blade type averaged across all treatments.

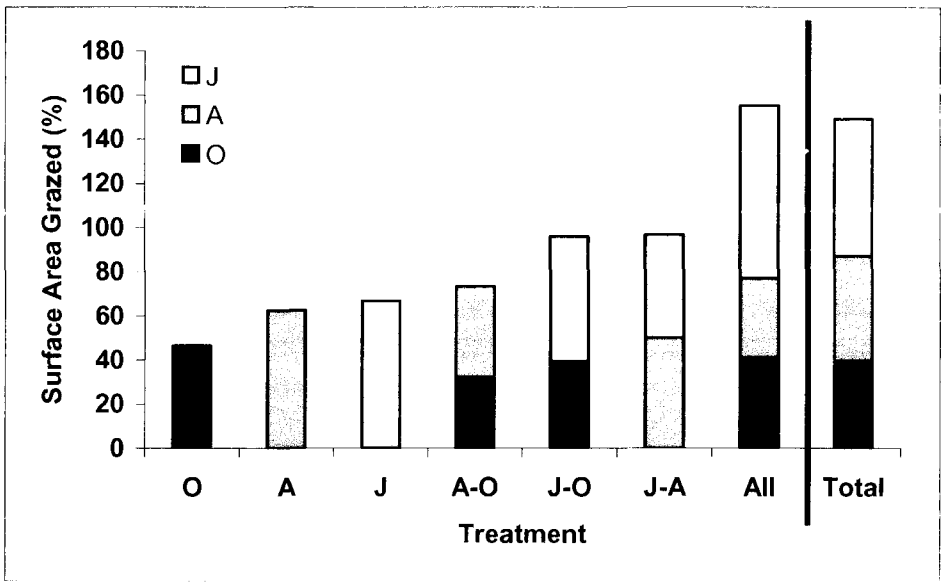

Figure 4.3: Surface area grazed for each blade age-class after 48 hours of grazing pressure by *Lacuna vincta*. Snails were offered either old (O), adult (A), or juvenile blades (J) separately, had the choice between O and A blades, O and J blades, or A and J blades, and were also offered all three blade types simultaneously (All) (n = 1 for each treatment). 'Total' represents the surface area grazed for each blade type averaged across all treatments. The surface are grazed of each blade type does not exceed 100%.

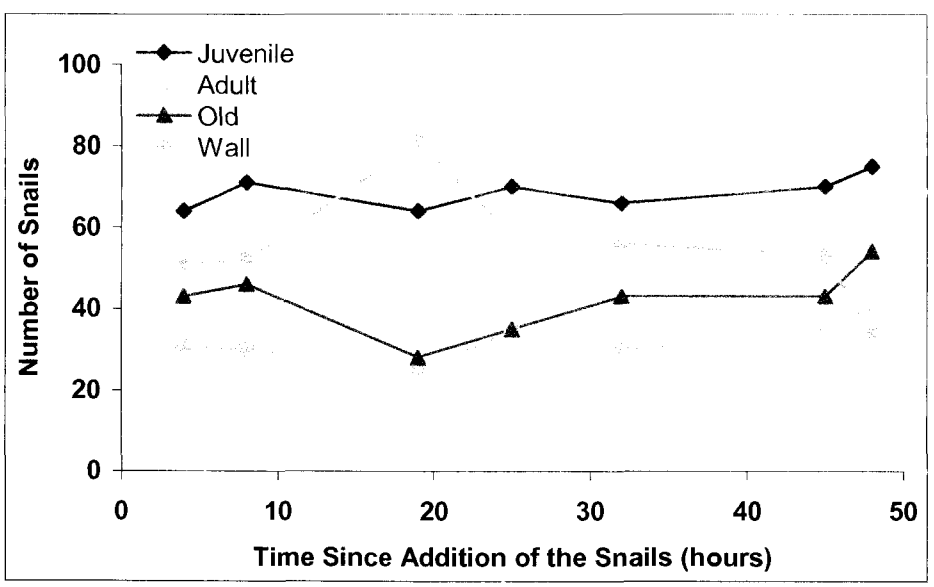

Figure 4.4: Snail abundance for each substrate type over 48 hours. The snails could either settle on old, adult, or juvenile blades, or on container walls. Snail counts on each substrate were done 4, 8, 19, 25, 32, 45, and 48 hours after the addition of the snails to the containers. A total of 200 snails (25 per container) were originally added but some snails escaped from the containers.

LITERATURE CITED

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CHAPTER 5
GENERAL CONCLUSIONS

This study suggests that estuarine factors of salinity and light attenuation, associated with turbidity, can influence the performance of *Nereocystis luetkeana*. The growth of oceanic *Nereocystis* sporophytes was reduced under estuarine conditions (a combination of low salinity and high turbidity) and certain aspects of *Nereocystis* spore development were negatively affected by reduced salinity. Results from the *in-situ* and laboratory experiments indicate that *Nereocystis* sporophytes and spores collected from different kelp beds responded differently to environmental factors, suggesting that individuals may be best adapted or acclimated to the conditions at the original site. It is, however, difficult to distinguish between ecotypes or phenotypes, as no data are available at this time to assess whether kelp beds in Kachemak Bay are genetically different.

This study also suggests that *Nereocystis* spores may be more sensitive to the environmental factors studied than sporophytes. Other ontogenic stages (gametogenesis, gamete formation, fertilization or differentiation into sporophytes) may have lower tolerance to estuarine conditions than spores and may provide further explanation on the absence of kelp beds in the inner bay. It is believed that additional factors, such as temperature, nutrient concentration, substrate character, wave and current regimes, acting independently and synergistically, and processes such as water circulation and spore dispersal are involved in the distribution of *Nereocystis* kelp beds in Kachemak Bay.

In addition to large-scale estuarine effects on sporophyte growth and spore development, grazing was found to be important at the local scale, in some study sites. The dynamics of kelp forests results from complex interactions between large-scale physical processes and local-scale biological processes. This study emphasizes the need to take into consideration both environmental and biological factors and both planktonic and benthic algal life stages to reach a better and more accurate understanding of the ecology and dynamics of *Nereocystis* kelp forests.

Kachemak Bay is one of the world's most productive marine ecosystems and shelters an important commercial and recreational fisheries industry. This critical ecosystem is still relatively pristine but it is receiving increasing pressure from a growing human population. As anthropogenic activities in and around the bay expand, risks of critical alterations to the ecosystem, such as increased sedimentation and turbidity and decreased salinity, also rise. A better understanding of factors and mechanisms that drive kelp forest dynamics is essential for sound resource management practices.